DNeasy® Blood & Tissue Handbook

DNeasy Blood & Tissue Kit DNeasy 96 Blood & Tissue Kit

For purification of total DNA from

animal blood

animal tissue

rodent tails

ear punches

cultured cells

fixed tissue

bacteria

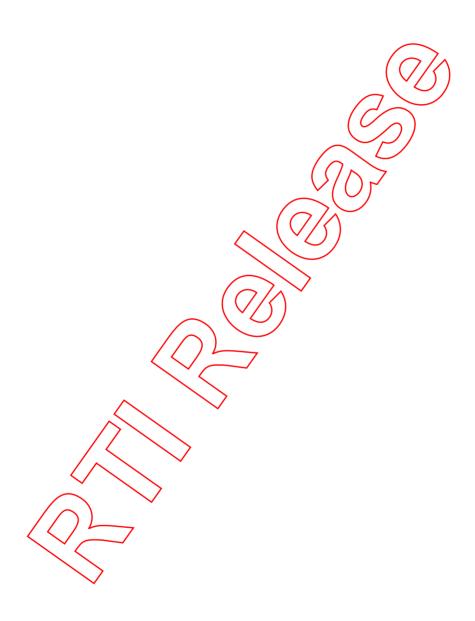
insects



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The PCR process is covered by the foreign counterparts of U.S. Patents Nos. 4,683,202 and 4,683,195 owned by F. Hoffmann-La Roche Ltd. QlAzol Lysis Reagent is a subject of US Patent No. 5,346,994 and foreign equivalents. "RNAlater" is a trademark of AMBION, Inc., Austin, Texas and is covered by various U.S. and foreign patents.

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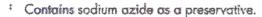
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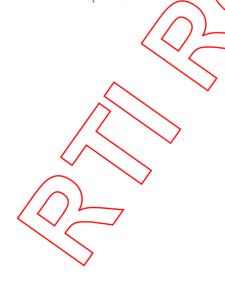
Kit Contents

DNeasy Blood & Tissue Kit	(50)	(250)
Catalog no.	69504	69506
Number of preps	50	250
DNeasy Mini Spin Columns (colorless)		
in 2 ml Collection Tubes	50	250
Collection Tubes (2 ml)	100	500
Buffer ATL	10 ml	50 ml
Buffer AL*	12 m	54 ml
Buffer AW1 (concentrate)*1	19 m	95 ml
Buffer AW2 (concentrate) ^{†‡}	12 mt	66 ml
Buffer AE	22 ml	2 x 60 ml
Proteinase K	(1,25 ml	6 ml
Handbook	VUT	j

^{*} Contains a chaotropic salt. Not compatible with disinfecting agents containing bleach. See page 8 for safety information.

[†] Buffer AW1 and Buffer AW2 are supplied as concentrates. Add sthanol (96–100%) according to the bottle label before use to obtain a working solution.





DNeasy 96 Blood & Tissue Kit	(A)	(12)
Calaing no.	69581	69582
Number of preps	4 n 96	12 x 96
DNeasy 96 Plates	4	12
S-Blocks*	2	2
Collection Microtubes, 1.2 ml (racked)	4 × 96	12 x 96
Collection Microtube Caps	2 x (120 x 8)	$5 \times (120 \times 8)$
Elution Microtubes RS (racked) and caps	4 x 96	12 × 96
AirPore Tape Sheets	25	3 x 25
Buffer AL ^t	86 ml	247 ml
Buffer ATL	80 ml	$3 \times 80 \text{ ml}$
Buffer AVV1 (concentrate) ¹¹	98 ml	$3 \times 98 \text{ ml}$
Buffer AW2 (concentrate) ¹⁵	08 ml	3 x 68 ml
Buffer AE	2x/110 ml	500 ml
Proteinase K	2 x 7 ml	$5 \times 7 \text{ ml}$
96-Well-Plate Register	4	12
Handbook) 1	1

^{*} Reusable; see Appendix B (page 54) for cleaning instructions.

Storage

DNeasy spin columns, DNeasy 96 plates, and all buffers should be stored dry, at room temperature (13–25°C) and are stable for 1 year under these conditions.

DNeasy Blood & Tissue Kits contain a ready-to-use proteinase K solution, which is supplied in a specially formulated storage buffer. Proteinase K is stable for at least 1 year after delivery when stored at room temperature. For storage longer than one year or if ambient temperatures often exceed 25°C, we suggest storing proteinase K at 2–8°C.

Contains a chaotropic salt. Not compatible with disinfectants containing bleach. See page 8 for safety information.

Buffer AW1 and Buffer AW2 are supplied as concentrates. Add ethanol (96-100%) according to the bottle label before use to obtain a working solution.

Contains sodium azide as a preservative.

Product Use Limitations

DNeasy Blood & Tissue Kits and DNeasy 96 Blood & Tissue Kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactority due to any reason other than misuse, QIAGEN will replace it free of charge or refune the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding DNeasy Blood & Tissue Kits or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of DNeasy Blood & Tissue Kits and DNeasy 96 Blood & Tissue Kits is tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffer AL and Buffer AW1 contain guanidine hydrachloride, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to components of DNeasy Blood & Tissue Kits and DNeasy 96 Blood & Tissue Kits.

Buffer AL and Buffer AW I (concentrate)

Contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* R22-36/38, S13-26-36-46

Proteinuse K

Contains proteinase K: sensitizer, irritant. Risk and safety phrases:* R36/37/38-42/43, S23-24-26-36/37

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R22: Harmful if swallowed; R36/37/38: Irritating to eyes, respiratory system and skin; R36/38: Irritating to eyes and skin; R42/43: May cause sensitization by inhalation and skin contact; S13: Keep away from food, drink, and animal feedingstuffs; S23: Do not breathe spray; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37: Wear suitable protective clothing and gloves; S46: If swallowed, seek medical advice immediately, and show container or label.

Introduction

DNeasy Blood & Tissue Kits are designed for rapid purification of total DNA (e.g., genomic, mitochondrial, and pathogen) from a variety of sample sources including fresh or frozen animal tissues and cells, blood, or bacteria. DNeasy purified DNA is free of contaminants and enzyme inhibitors and is highly suited for PCR, Southern blotting, RAPD, AFLP, and RFLP applications.

Purification requires no phenol or chloroform extraction or alcohol precipitation, and involves minimal handling. This makes DNeasy Blood & Tissue Kits highly suited for simultaneous processing of multiple samples. For higher-throughput applications, the DNeasy 96 Blood & Tissue Kit enables simultaneous processing of 96 or 192 samples.

The buffer system is optimized to allow direct cell lysis followed by selective binding of DNA to the DNeasy membrane. After lysis, the DNeasy Blood & Tissue spin-column procedure can be completed in as little as 20 minutes. Using the DNeasy 96 Blood & Tissue Kit, 96 or 192 samples can be processed in just 1 hour after lysis.

Simple centrifugation processing completely removes conteminants and enzyme inhibitors such as proteins and divalent cations, and allows simultaneous processing of multiple samples in parallel. In addition, DNeasy Blood & Tissue procedures are suitable for a wide range of sample sizes.

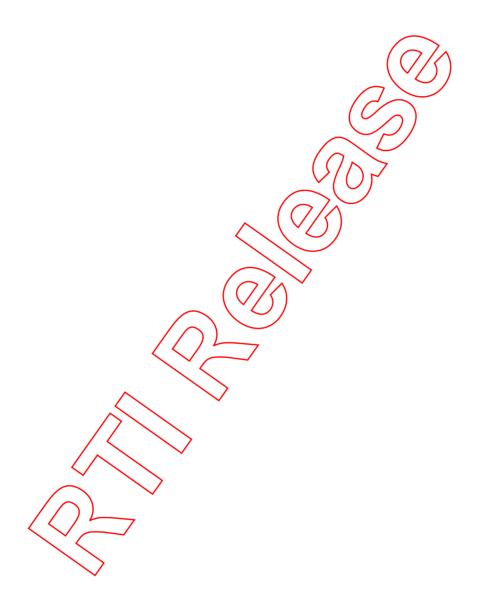
Purified DNA is eluted in low-salt buffer or water, ready for use in downstream applications. DNeasy purified DNA typically has an A_{260}/A_{280} ratio between 1.7 and 1.9, and is up to 50 kb in size, with fragments of 30 kb predominating. The DNeasy procedure also efficiently recovers DNA fragments as small as 100 bp.

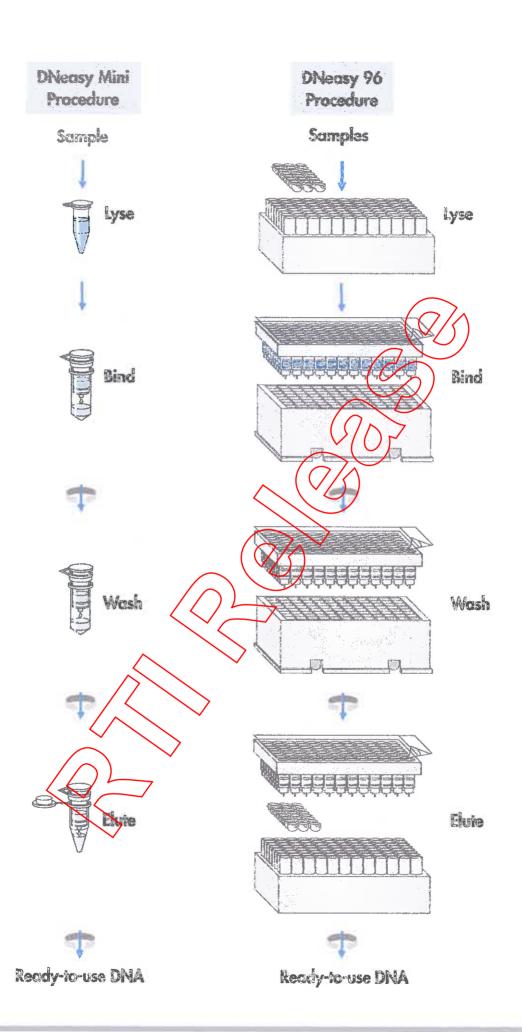
Principle and procedure

DNeasy Blood & Tissue procedures are simple (see flowchart). Samples are first lysed using proteinase K.* Buffering conditions are adjusted to provide optimal DNA-binding conditions and the lysate is loaded onto the DNeasy Mini spin column or the DNeasy 96 plate. During centrifugation, DNA is selectively bound to the DNeasy membrane as contaminants pass through. Remaining contaminants and enzyme inhibitors are removed in two efficient wash steps and DNA is then eluted in water or buffer, ready for use. DNeasy purified DNA has A_{260}/A_{280} ratios of 1.7–1.9, and absorbance scans show a symmetric peak at 260 nm confirming high purity.

^{*} Lysis efficiency can be improved by cell disruption using a rotor-stator homogenizer, such as the QIAGEN TissueRuptor, or a bead mill, such as the QIAGEN TissueLyser. A supplementary protocol allowing the simultaneous disruption of up to 48 tissue samples using the QIAGEN TissueLyser is available from QIAGEN Technical Services.

The DNeasy membrane combines the binding properties of a silica-based membrane with simple microspin technology or with the QIAGEN 96-Well-Plate Centrifugation System. DNA adsorbs to the DNeasy membrane in the presence of high concentrations of chaotropic salt, which remove water from hydrated molecules in solution. Buffer conditions in DNeasy Blood & Tissue procedures are designed to enable specific adsorption of DNA to the silica membrane and optimal removal of contaminants and enzyme inhibitors.





Description of protocols

Different protocols in this handbook provide detailed instructions to use DNeasy Kits for purification of total DNA.

The protocol "Purification of Total DNA from Animal Blood or Cells (Spin-Column Protocol)", page 25, is for use with the DNeasy Blood & Tissue Kit, for purification of DNA from animal blood (with nucleated or nonnucleated erythrocytes) or from cultured animal or human cells.

The protocol "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)", page 28, is for use with the DNeasy Blood & Tissue Kit, for purification of DNA from animal tissues, including rodent tails.

The protocol "Purification of Total DNA from Animal Blood or Cells (DNeasy 96 Protocol)", page 31, is for use with the DNeasy 96 Blood & Tissue Kit, for high-throughput purification of DNA from animal blood (with nucleated or nonnucleated erythrocytes) or from cultured animal or human cells.

The protocol "Purification of Total DNA from Avintal Tissues (DNeasy 96 Protocol)", page 35, is for use with the DNeasy 96 Blood & Tissue Kit, for high-throughput purification of DNA from animal tissues, including radent tails.

Pretreatment and specialized protocols

There are several pretreatment protocols included in this handbook, which are optimized for specific sample types. These pretreatment protocols are used in conjunction with one of the DINA purification protocols described above.

The following pretreatment protocols are included in this handbook.

- Pretreatment for Paraffin-Embedded Tissue, page 41
- Pretreatment for Formalin-Fixed Tissue, page 43
- Pretreatment for Gram-Negative Bacteria, page 44
- Pretreatment for Gram-Positive Bacteria, page 45

Additional optimized protocols for purification of DNA from yeast, hair, insects, crude lysates, bone, saliva, and other specialized sample types are available online at www.qiagen.com/literature/protocols/DNeasyTissue.aspx or from QIAGEN Technical Services (see back cover).

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

For all protocols

- Pipets and pipet tips
- Vortexer
- Ethanol (96–100%)*
- Optional: RNase A (100 mg/ml; cat. no. 19101)

For DNeusy Blood & Tissue Kit (spin column) protocols

- Microcentrifuge tubes (1.5 ml or 2 ml)
- Microcentrifuge with rotor for 1.5 ml and 2 ml tubes
- Thermomixer, shaking water bath, or rocking platform for heating at 56°C

For DNeasy 96 Blood & Tissue Kit protocols

- Centrifuge 4-15C or 4K15C with Plate Rotor 2x 96 (see page 20)
- Multichannel pipet with extended tips

 For efficient processing, we recommend the use of an electric multichannel pipet with a capacity of at least 1 ml pet pipet tip. Options include the Matrix Impact® cordless electronic multichannel pipet, which has a unique, adjustable tip-spacing system allowing the user to transfer liquid directly from sample tubes to 96-well plates.

We recommend using extended tips with a maximum volume of 1250 µl with the Matrix multichannel pipet (available from Matrix, cat. no. 8255 for tips with filters or 8252 for tips without filters).

These multichannel pipets and pipet tips can be purchased from Matrix Technologies Corporation (www.matrixtechcorp.com).[†]

- Reagent reservoirs for multichannel pipets
- Heavy plate or similar object to place on top of collection microtubes during incubation
- Oven or incubator for heating at 56°C
- * Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
- [†] This is not a complete list of suppliers and does not include many important vendors of biological supplies.

For blood and cultured cells

PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl)

For pretreatment of paraffin-embedded tissue (page 41)

Xylene

For prefreatment of formalin-fixed tissue (page 43)

PBS, pH 7.2 (50 mM potassium phosphate, 150 mM NaCl)

For pretreatment of gram-positive bacteria (page 45)

- Enzymatic lysis buffer:
 - 20 mM Tris·Cl, pH 8.0
 - 2 mM sodium EDTA
 - 1.2% Triton® X-100
 - Immediately before use, add lysozyme to 20 mg/ml

Important Notes

Sample collection and storage

Best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -70°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor-quality starting material will also lead to reduced length and yield of purified DNA.

After proteinase K digestion, tissue samples can also be stored in Buffer ATL for up 6 months at ambient temperature without any reduction in DNA quality.

For certain bacterial cultures that accumulate large amounts of metabolites and/or form very dense cell walls, it is preferable to harvest cells in the early log phase of growth. Fresh or frozen cell pellets can be used in the procedure.

Starting amounts of samples

DNeasy Blood & Tissue procedures give DNA yields that increase linearly over a wide range, for both very small and large sample sizes (e.g., from as little as 100 cells up to $5 \times 10^6 \text{ cells}$).

Maximum amount of starting material

In order to obtain optimum DNA yield and quality, it is important not to overload the DNeasy spin column or DNeasy 96 plate as this can lead to significantly lower yields than expected (see Figure 1). For samples with very high DNA contents (e.g., spleen, which has a high cell density, and cell lines with a high degree of ploidy), less than the recommended amount of sample listed in Table 1 should be used. If your starting material is not shown in Table 3 (page 23) and you have no information regarding DNA content, we recommend beginning with half the maximum amount of starting material indicated in Table 1. Depending on the yield obtained, the sample size can be increased in subsequent preparations.

Very small comple sizes

DNeasy Blood & Tissue procedures are also suitable for purifying DNA from very small amounts of starting material. If the sample has less than 5 ng DNA (<10,000 copies), 3–5 µg carrier DNA (a homopolymer such as poly-dA, poly-dT, or gDNA) should be added to the starting material. Ensure that the carrier DNA does not interfere with your downstream application. In order to prevent any interference of the carrier with the downstream application, an RNA carrier can be used. This can be removed later by RNase digestion. DNA or RNA homopolymers can be purchased from various suppliers.

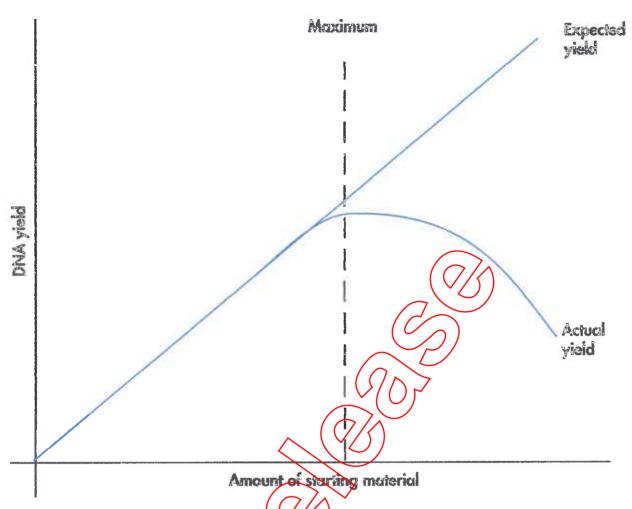


Figure 1 Schematic diagram of effect of sample size on DNA yield. If more than the maximum amount of starting material is used, DNA yield will be lower than expected.

Table 1. Maximum Amounts of Starting Material

Sample	Amount
Animal tissue (see Table 3, page 23)	25 mg (spin-column protocols)
	20 mg (DNeasy 96 protocols)
Mammalian blood (see Table 4, page 23)	100 pl
Bird or tish blood (with nucleated erythrocytes)	ابا 10
Mouse tail	0.6–1.2 cm
Rat tail	0.6 cm
Cultured cells	5 x 10°
Bacteria	2 x 10°

Quantification of starting material

Weighing tissue or counting cells is the most accurate way to quantify starting material. However, the approximate guidelines given below can also be followed.

Animol lissue

A 2 mm cube (approximately this size: M; volume, approximately 8 mm³) of most animal tissues weighs approximately 10–15 mg.

Animal cells

The number of HeLa cells obtained in various culture dishes after confluent growth is given in Table 2.

Table 2. Growth Area and Number of Hela Cells in Various Culture Dishes

Cell culture vessel	Growth area (lan	Number of cells [†]
Multiwell plates		
96-well	0.32-0.6	4–5 x 10⁴
48-well	1	1 x 10 ⁵
24-well	2	2.5 x 10 ⁵
12-well		5 x 10 ⁵
6-well	2.5	1 x 10°
Dishes		
35 mm	8	1 x 10°
60 mm	21	2.5 x 10°
100 mm	V 56	7 x 10°
145–150 mm) 145	2×10^{7}
Flesks		
40–50 ml	25	3 x 10°
250–300 ml	75	1×10^{7}
650-750 ml	162–175	2×10^7

^{*} Per well, if multiwell plates are used; varies slightly depending on the supplier.

Cell numbers given are for HeLa cells (approximate length = 15 µm) assuming confluent growth. Cell numbers vary since animal cells can vary in length from 10 to 100 µm.

Bacteria

Bacterial growth is usually measured using a spectrophotometer. However, it is very difficult to give specific and reliable recommendations for the correlation between OD values and cell numbers in bacterial cultures. Cell density is influenced by a variety of factors (e.g., species, media, and shaker speed) and OD readings of cultures measure light scattering rather than absorption. Measurements of light scattering are highly dependent on the distance between the sample and the detector and therefore readings vary between different types of spectrophotometer. In addition, different species show different OD values at defined wavelengths (e.g., 600 or 436 nm).

We therefore recommend calibrating the spectrophotometer used by comparing OD measurements at appropriate wavelengths with viable cell densities determined by plating experiments (e.g., see Ausubel, F.M. et al., eds. [1997] Current Protocols in Molecular Biology, New York: John Wiley & Sons, Inc.). OD readings should be between 0.05 and 0.3 to ensure significance. Samples with readings above 0.3 should be diluted so that the readings fall within this range and the dilution factor used in calculating the number of cells per milliliter.

The following calculation can be considered as a rough guide, which may be helpful. An E. coli culture of 1 x 10° cells per milliliter, diluted 1 in 4, gives OD600 values of 0.25 measured using a Beckman DU-7400 or 0.125 using a Beckman DU-40 spectrophotometer. These correspond to calculated OD values of 1.0 or 0.5 respectively for 1 x 10° cells per milliliter.

Preparation of Buffer AWI and Buffer AW2

Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle and shake thoroughly. Buffer AW1 and Buffer AW2 are stable for at least 1 year after the addition of ethanol when stored closed at room temperature (15–25°C).

Buffer AL

Purification of DNA from animal blood, cultured cells, or Gram-positive bacteria

Buffer AL must be added to the sample and incubated at 56°C before ethanol is added. Ensure that ethanol has not been added to Buffer AL beforehand. Buffer AL can be purchased separately (see page 56 for ordering information).

Purification of DNA from animal tissues

Buffer AL and ethanol (96–100%) are added in the same step. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

For the protocol "Purification of Total DNA from Animal Tissues (DNeasy 96 Protocol)": Add 90 ml ethanol (96–100%) to the bottle containing 86 ml Buffer AL or 260 ml ethanol to the bottle containing 247 ml Buffer AL and shake thoroughly. Mark the bottle to indicate that ethanol has been added. (Please note that, for purification of DNA from animal blood, Buffer AL must be used without ethanol. Buffer AL can be purchased separately if the same kit will be used for purification of DNA from animal blood.)

Buffer AL is stable for 1 year after the addition of ethanol when stored closed at room temperature.

Profeinase K

DNeasy Blood & Tissue Kits contain ready-to-use proteinase K supplied in a specially formulated storage buffer. The activity of proteinase K is 600 mAU/ml solution (or 40 mAU/mg protein), and has been chosen to provide optimel results.

Also included in the kits is an optimized buffer for tissue lysis. Buffer ATL. To enable efficient lysis, it is advisable to cut animal tissue into small pieces. If desired, lysis time can be reduced to 20 minutes by grinding the sample in liquid nitrogen* before addition of Buffer ATL and proteinase K. Alternatively, tissue samples can be effectively disrupted before proteinase K digestion using a rotor-stator homogenizer, such as the QIAGEN TissueRuptor, or a bead mill, such as the QIAGEN TissueLyser. A supplementary protocol for simultaneous disruption of up to 48 tissue samples using the TissueLyser can be obtained by contacting QIAGEN Technical Services (see back cover).

Proteinase K is stable for at least one year after delivery when stored at room temperature (15–25°C). To store for more than one year or if ambient temperature often exceeds 25°C, we suggest keeping proteinage K at 2–8°C.

Please contact QIAGEN Technical Services or your local distributor if you have any questions about the use of proteinase K (see back cover).

Copurification of ANA

DNeasy Blood & Tissue Kits copurify DNA and RNA when both are present in the sample. Transcriptionally active tissues such as liver and kidney contain high levels of RNA, which will be sopurified. RNA may inhibit some downstream enzymatic reactions, although it does not affect PCR. If RNA-free genomic DNA is required, RNase A should be added to the sample before addition of Buffer AL, to digest the RNA. DNeasy protocols describe the use of an RNase A stock solution of 100 mg/ml. However, the amounts of RNase can be adjusted with less concentrated stock solutions, but not more than 20 µl of RNase solution should be used. Refer to the protocols for details.

^{*} When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Centrifugation (DNeasy 96 procedures)

Centrifuges 4-15C and 4K15C

DNeasy 96 spin protocols use a streamlined centrifugation procedure that enables purification of DNA from up to 2×96 samples in parallel for direct use in any downstream application. The DNeasy 96 Blood & Tissue procedure requires use of the QIAGEN 96-Well-Plate Centrifugation System, comprising the Plate Rotor 2×96 and the table-top Centrifuge 4-15C or the refrigerated table-top Centrifuge 4K15C (see page 55 for ordering information). In addition to the Plate Rotor 2×96 , a wide range of other rotors can be used with these centrifuges.

Standard table-top centrifuges and microtiter plate rotors are not suitable for the DNeasy 96 protocol for 2 reasons: the microtiter plate buckets are either not deep enough for the complete DNeasy 96 package or they will not swing out properly, and furthermore, high g-forces (> $5500 \times g$) are required for optimal performance of the DNeasy 96 procedure. The speed limit of the Centrifuge 4-15C and the Centrifuge 4K15C (6000 rpm; $5796 \times g$) is programmed so that the given g-force will not be exceeded. All centrifugation steps are performed at room temperature.

MPORTANT: Centrifuges must be properly maintained for optimal performance. It is particularly important that the buckets and rotor pins are routinely greased to prevent suboptimal running conditions that may lead to cracking of DNeasy 96 plates.

For further information about QIAGEN Centrifuges and the Plate Rotor 2 x 96, contact QIAGEN Technical Services or your tocal distributor (see back cover for contact information).

Note: If the Centrifuge 4K15C is used, set the temperature to 40°C for all centrifugation steps.

Note: Use AirPore Tape Sheets (provided) to seal DNeasy 96 plates during all centrifugation steps to prevent cross-contamination between samples.

Abbreviated instructions for using the Centrifuge 4-15C and Centrifuge 4K15C

Warning: Never run the centrifuge with empty plate carriers placed inside the buckets, that is, without the collection microtubes or DNeasy 96 plates and S-Blocks. If unsupported, the carriers will callapse under high g-forces. Therefore, remove the carriers during test runs. Standard microtiter plates may be centrifuged in the same carriers if the g-force does not exceed $500 \times g$.

- 1. Switch on the centrifuge by pressing the main switch on the back.
- 2. Select the rotor selection list in the display field by turning the knob. After pressing the knob, turn the knob again to select the rotor/bucket combination "09100/09158" for the Plate Rotor 2 x 96. Confirm entry by pressing the knob. Entering the rotor number automatically sets the time and speed limits for centrifugation for that particular rotor, thus eliminating the danger of the centrifuge over-speeding.

- 3. Select "Speed" by turning the knob. Press the knob and by turning the knob again, set the speed to "6000". Confirm entry by pressing the knob. The corresponding relative centrifugal force (RCF) is calculated from the rotor number and speed and appears automatically in the RCF field. It is also possible to enter the RCF value "5796 x g" manually in the RCF field after selecting "RCF" in the same way.
- 4. Select "Time" by turning the knob. Press once and by turning the knob again, set the time as recommended in the particular protocol step. Confirm entry by pressing the knob.
- 5. For the Centrifuge 4K15C, set the temperature to 40°C.
- 6. Open the lid, place the 96-well plates with the metal carriers in the buckets then close the lid. The start and lid keys light up.
- 7. Push "Start" to start the centrifuge. When the centrifuge is running the lid key will not be lit. Each run can be interrupted by pushing "Stop"
- 8. At the end of the run, the lid key will light up. Open the centrifuge lid by pressing the lid key. Remove the plates. All preset parameters remain after a run has finished.

Elution of pure nucleic acids

Purified DNA is eluted from the DNeasy Mini spin column or DNeasy 96 plate in either Buffer AE or water. For maximum DNA yield, elution is performed in two successive steps using 200 µl Buffer AE each. For ware concentrated DNA, elution can be performed in two successive steps of 100 µl each. Keep in mind that elution volume and number of elution steps depends on the amount of DNA bound to the DNeasy membrane (see Figure 2).

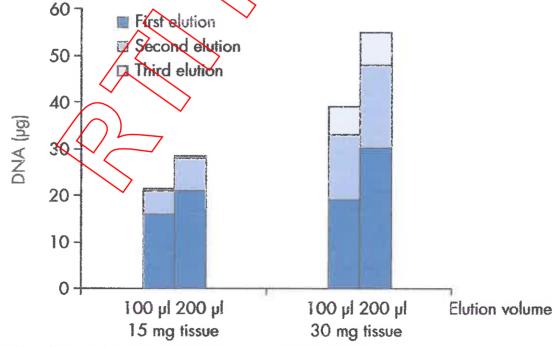


Figure 2 Yields of total nucleic acids in successive elutions of 100 or 200 µl.

For samples containing up to 10 μ g DNA, a single elution step using 200 μ l is sufficient. For samples containing more than 10 μ g DNA, a second elution step with another 200 μ l Buffer AE is recommended. Approximately 60–80% of the DNA will elute in the first elution. If >30 μ g DNA is bound to the DNeasy membrane, elution in 3 x 200 μ l may increase yield (see below).

Elution in 100 µl increases the DNA concentration in the eluate, but reduces overall DNA yield. To prevent dilution of the first eluate, the subsequent elution step can be performed using a fresh 1.5 ml microcentrifuge tube. More than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.

For very small samples (containing less than 1 µg DNA), only one elution in 50 µl of Buffer AE or water is recommended.

Buffer AE is 10 mM Tris·Cl, 0.5 mM EDTA, pH 9.0. Elation with Buffer AE guarantees optimal recovery and stability of eluted DNA. However, if you wish to elute DNA with water please ensure that the pH of the water is at least 7.0 deionized water from certain sources can be acidic). For long-term storage of DNA, elution in Buffer AE is strongly recommended since DNA stored in water is subject to acid hydrolysis. Buffer AE should be used at room temperature (15–25°C). Heating Buffer AE before elution is not necessary.

Expected yields

Yields of genomic DNA will vary from sample to sample depending on the amount and type of material processed. In addition, the quality of starting material will affect DNA yield.

Tables 3 and 4 can be used to provide an estimate of expected yield.

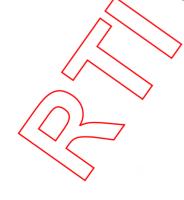


Table 3. Typical DNA Yields from Animal Tissues Using DNeasy Blood & Tissue Kits

Source	Amount	DNA (µg)
Mammalian blood (see Table 4)	100 թl	3–6
Bird blood	5 µl	9-40
Lymphocytes	5 x 10°	15-25
HeLa cells	2 x 10°	15–25
Liver	25 mg	10-30
Brain	25 mg	15-30
Lung	25 mg	5-10
Heart	25 mg	5-10
Kidney	25 mg	1530
Spleen	10 mg	5–30
Mouse tail	1.2 cm (tip)	10–25
Rat tail	0.0 cm (Hp)	20-40
Pig ear	(23 mg	10–30
Horse hair	10 hairs	2-4
Fish fin	20 mg	10–20
Fish spawn (mackerel)	() 10 mg	5-10

Table 4. Typical DNA Yields from Animal Blood Using DNeasy Blood & Tissue Kits

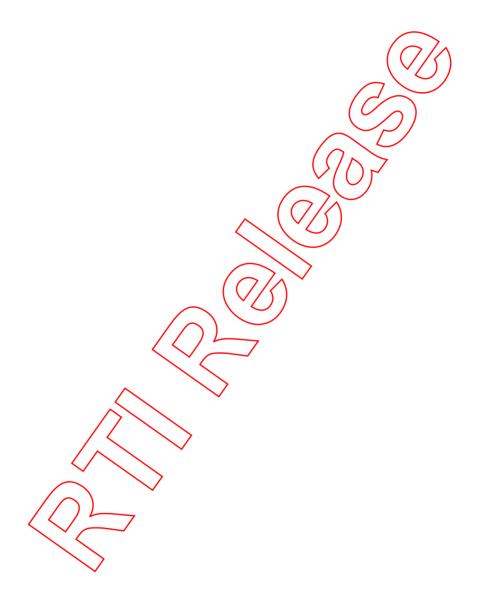
Animal	Amount (µl)	DNA (µg)
Cattle	100	4-5
Horse	100	3-5
Pig	100	3-6
Sheep	100	3–6
Dog	100	4-5
Cat	100	3–6
Goat	50*	3
Chicken [†]	.5	9-15

^{*} Using more than 50 µl goat blood gave no significant increase in DNA yield.

¹ Bird blood contains nucleated erythrocytes, giving higher DNA yields than mammalian blood.

Purification of high-molecular-weight DNA

QIAGEN Genomic-tips and Blood & Cell Culture DNA Kits are recommended for large-scale purification of high-molecular-weight DNA (see page 56 for ordering information). QIAGEN Genomic-tips are available for purification of up to 500 µg of genomic DNA ranging in size from 50 to 150 kb. They are highly suited for use in Southern blotting, library construction, genome mapping, and other demanding applications. Please contact QIAGEN Technical Services or your local distributor for more information (see back cover).



Protocol: Purification of Total DNA from Animal Blood or Cells (Spin-Column Protocol)

This protocol is designed for purification of total DNA from animal blood (with nucleated or nonnucleated erythrocytes) or from cultured animal or human cells.

Important points before starting

- If using the DNeasy Blood & Tissue Kit for the first time, read "Important Notes" (page 15).
- All centrifugation steps are carried out at room temperature (15-25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5-10 s.
- PBS is required for use in step 1 (see page 14 for composition). Buffer ATL is not required in this protocol.
- Optional: RNase A may be used to digest RNA during the procedure. RNase A is not provided in the DNeasy Blood & Tissue Kit (see "Copurification of RNA", page 19).

Things to do before starting

- Buffer AL may form a precipitate upon storage. If necessary, warm to 56°C until the precipitate has fully dissolved.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a thermomixer, shaking water bath, or rocking platform to 56°C for use in step 2.

Procedure

- 1. For blood with nonnucleated erythrocytes, follow step 1 a; for blood with nucleated erythrocytes, follow step 1 b; for cultured cells, follow step 1 c.
 - Blood from mammals contains nonnucleated erythrocytes. Blood from animals such as birds, fish, or frogs contains nucleated erythrocytes.
- 1a. Nonnucleated: Pipet 20 µl proteinase K into a 1.5 ml or 2 ml microcentrifuge tube (not provided). Add 50-100 µl anticoagulated blood. Adjust the volume to 220 µl with PBS. Continue with step 2.
 - Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature before continuing with step 2.

- 1b. Nucleated: Pipet 20 µl proteinase K into a 1.5 ml or 2 ml microcentrifuge tube (not provided). Add 5-10 µl anticoagulated blood. Adjust the volume to 220 µl with PBS. Continue with step 2.
 - Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature before continuing with step 2.
- 1c. Cultured cells: Centrifuge the appropriate number of cells (maximum 5 x 10°) for 5 min at 300 x g. Resuspend the pellet in 200 µl PBS. Add 20 µl proteinase K. Continue with step 2.

When using a frozen cell pellet, allow cells to thaw before adding PBS until the pellet can be dislodged by gently flicking the tube.

Ensure that an appropriate number of cells is used in the procedure. For cell lines with a high degree of ploidy (e.g., HeLa cells), it is recommended to use less than the maximum number of cells listed in Table 1, page 16.

Optional: If RNA-free genomic DNA is required, add 4 µ/RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing with step 2.

- 2. Add 200 pl Buffer AL (without added extends). Mix thoroughly by vortexing, and incubate at 56°C for 10 min.
 - Ensure that ethanol has not been added to Buffer AL (see "Buffer AL", page 18). Buffer AL can be purchased separately (see page 56 for ordering information).
 - It is essential that the sample and Buffer AL are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.
- 3. Add 200 µl ethanol (% 100%) to the sample, and mix thoroughly by vortexing. It is important that the sample and the ethanol are mixed thoroughly to yield a homogeneous solution.
- 4. Pipet the mixture from step 3 into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at ≥6000 x g (8000 rpm) for 1 min. Discard flow-through and collection tube.*
- 5. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AWI, and centrifuge for 1 min at ≥6000 x g (8000 rpm). Discard flow-through and collection tube.°
- 6. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.
 - It is important to dry the membrane of the DNeasy Mini spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.
- * Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 8 for safety information.

Following the centrifugation step, remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at $20,000 \times g$ (14,000 rpm).

7. Place the DNeasy Mini spin column in α clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200 μl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥6000 x g (8000 rpm) to elute.

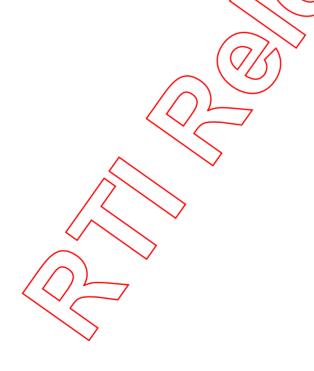
Elution with 100 µl (instead of 200 µl) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield (see Figure 2, page 21).

8. Recommended: For maximum DNA yield, repeat elution once as described in step 7.

This step leads to increased overall DNA yield.

A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluctes, the microcentrifuge tube from step 7 can be reused for the second elution/step.

Note: Do not elute more than 200 µl into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate.



Protocol: Purification of Total DNA from Animal Tissues (Spin-Column Protocol)

This protocol is designed for purification of total DNA from animal tissues, including rodent tails.

Important points before starting

- If using the DNeasy Blood & Tissue Kit for the first time, read "Important Notes" (page 15).
- For fixed tissues, refer to the pretreatment protocols "Pretreatment for Paraffin-Embedded Tissue", page 41, and "Pretreatment for Formalin-Fixed Tissue", page 43.
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5-10 s.
- Optional: RNase A may be used to digest RMA during the procedure. RNase A is not provided in the DNeasy Blood & Tissue Kit (see "Copurification of RNA", page 19).

Things to do before starting

- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C until the precipitates have fully dissolved.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a thermomixer, shaking water bath, or rocking platform to 56°C for use in step 2.
- If using frozen tissue, equilibrate the sample to room temperature. Avoid repeated thawing and freezing of samples since this will lead to reduced DNA size.

Procedure

Cut up to 25 mg tissue (up to 10 mg spleen) into small pieces, and place in a 1.5 ml microcentrifuge tube. For rodent tails, place one (rat) or two (mouse) 0.4-0.6 cm lengths of tail into a 1.5 ml microcentrifuge tube. Add 180 µl Buffer ATL. Earmark the animal appropriately.

Ensure that the correct amount of starting material is used (see "Starting amounts of samples", page 15). For tissues such as spleen with a very high number of cells for a given mass of tissue, no more than 10 mg starting material should be used.

We strongly recommend to cut the tissue into small pieces to enable more efficient lysis. If desired, lysis time can be reduced by grinding the sample in liquid nitrogen* before addition of Buffer ATL and proteinase K. Alternatively, tissue samples can be effectively disrupted before proteinase K digestion using a rotor–stator homogenizer, such as the QIAGEN TissueRuptor, or a bead mill, such as the QIAGEN TissueLyser (see page 56 for ordering information). A supplementary protocol for simultaneous disruption of up to 48 tissue samples using the TissueLyser can be obtained by contacting QIAGEN Technical Services (see back cover).

For rodent tails, a maximum of 1.2 cm (mouse) or 0.6 cm (rat) tail should be used. When purifying DNA from the tail of an adult mouse or rat, it is recommended to use only 0.4–0.6 cm.

2. Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water both, or or racking platform. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h or, for rodent tails, 6–8 h. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.

After incubation the lysate may appear viscous, but should not be gelatinous as it may clog the DNeasy Mini spin column. If the lysate appears very gelatinous, see the "Troubleshooting Guide", page 47, for recommendations.

Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing with step 3.

Transcriptionally active tissues such as liver and kidney contain high levels of RNA, which will copurify with genomic DNA. For tissues that contain low levels of RNA, such as rodent tails, or if residual RNA is not a concern, RNase A digestion is not necessary.

3. Vortex for 15 s. Add 260 µl Buffer At to the sample, and mix thoroughly by vortexing. Then add 208 µl ethanol (96-100%), and mix again thoroughly by vortexing.

It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

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^{*} When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the DNeasy procedure. Some tissue types (e.g., spleen, lung) may form a gelatinous lysate after addition of Buffer AL and ethanol. In this case, vigorously shaking or vortexing the preparation is recommended.

- 4. Piper the mixture from step 3 (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at ≥6000 x g (8000 rpm) for 1 min. Discard flow-through and collection tube.*
- 5. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 pl Buffer AW1, and centrifuge for 1 min at ≥6000 x g (6000 rpm). Discard flow-through and collection tube.*
- 6. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x/g)(1/4,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.

It is important to dry the membrane of the DNeasy Mini spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the following elution.

Following the centrifugation step, remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through, since this will result in carryover of ethanol. If carryover of ethanol occurs, empty the collection tube, then reuse it in another centrifugation for 1 min at 20,000 x g (14,000 rpm).

7. Place the Diversy Mini spin columnia a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200 xl Bailer AE directly onto the Diversy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥6000 x g (8000 rpm) to elute.

Elution with 100 µl (instead of 200 µl) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield (see Figure 2, page 21).

8. Recommended: For maximum DNA yield, repeat elution once as described in step 7.

This step leads to increased overall DNA yield.

A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, to combine the eluates, the microcentrifuge tube from step 7 can be reused for the second elution step.

Note: Do not elute more than 200 µl into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate.

^{*} Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 8 for safety information.

Protocol: Purification of Total DNA from Animal Blood or Cells (DNeasy 96 Protocol)

This protocol is designed for high-throughput purification of total DNA from animal blood (with nucleated or nonnucleated erythrocytes) or from cultured animal or human cells.

Important points before starting

- If using the DNeasy 96 Blood & Tissue Kit for the first time, read "Important Notes" (page 15).
- All centrifugation steps are carried out at room temperature (15-25°C).
- PBS is required for use in step 1 (see page 14 for composition). Butter ATL is not required in this protocol.
- Ensure that ethanol has not been added to Buffer AL see "Important Notes", page 15). Buffer AL can be purchased separately (see page 56 for ordering information).
- Optional: RNase A may be used to digest RNA during the procedure. RNase A is not provided in the DNeasy 96 Blood & Tissue Kit (see "Copurification of RNA", page 19).

Things to do before starting

- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C for 5 min until the precipitates have fully dissolved.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Mix Buffer AW1 before use by inverting several times.
- Preheat an incubator to 55°C for use in step 2.

Procedure

- 1. For blood with nonnucleated erythrocytes, follow step 1 a; for blood with nucleated erythrocytes, follow step 1 b; for cultured cells, follow step 1 c.
 - Blood from mammals contains nonnucleated erythrocytes. Blood from animals such as birds, fish, or frogs contains nucleated erythrocytes.
- 1a. Nonnucleated: Pipet 20 µl proteinuse K into each collection microtube. Add 50-100 µl anticoagulated blood per collection microtube. Use a 96-Well-Plate Register (provided) to identify the position of each sample. Adjust the volume to 220 µl each with PBS. Continue with step 2.

Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) and incubate for 5 min at room temperature before continuing with step 2.

Keep the clear covers from the collection microtube racks for use in step 3.

1b. Nucleated: Pipet 20 µl proteinase K into each collection microtube. Add 5-10 µl anticoagulated blood. Use a 96-Well-Plate Register (provided) to identify the position of each sample. Adjust the volume to 220 µl each with PBS. Continue with step 2.

Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml) and incubate for 5 min at room temperature before continuing with step 2.

Keep the clear covers from the collection microtube racks for use in step 3.

1c. Cultured cells: Centrifuge the appropriate number of cells (moximum 5 x 10° each) for 5 min at 300 x g. Use a 96-Well-Plate Register (provided) to identify the position of each sample. Resuspend the pellets in 200 pl PES cerch. Add 20 pl proteinase K each. Continue with step 2.

When using a frozen cell pellets, allow cells to thow before adding PBS until the pellet can be dislodged by gently flicking the tube.

Ensure that an appropriate number of cells is used in the procedure. For cell lines with a high degree of ploidy (e.g., Hella cells), it is recommended to use less than the maximum number of cells listed in Table 1, page 16.

Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml). Seal the collection microtubes properly using the caps provided, mix by vortexing, and incubate for 5 min at room temperature before continuing with step 2.

Keep the clear covers from the collection microtube racks for use in step 3.

2. Add 200 pl Buffer AL (without added ethanol) to each sample.

Ensure that ethanol has not been added to Buffer AL (see "Buffer AL", page 18). Buffer AL can be purchased separately (see page 56 for ordering information).

3. Seal the collection microtobes properly using the caps provided. Place a clear cover (saved from step 1) over each rack of collection microtubes, and shake the racks vigorously up and down for 15 s. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.

Do not prolong this step.

IMPORIANT: The rack of collection microtubes must be vigorously shaken up and down with both hands to obtain a homogeneous lysate. Inverting the rack of collection microtubes is not sufficient for mixing. The genomic DNA will not be sheared by vigorous shaking. The lysate and Buffer AL should be mixed immediately and thoroughly to yield a homogeneous solution.

Keep the clear covers from the collection microtube racks for use in step 6.

 Incubate at 56°C for 10 min. Place a weight on top of the caps during the incubation. Mix accessionally during incubation to disperse the sample, or place on a rocking platform.

Note: Do not use a rotary- or vertical-type shaker as continuous rotation may release the caps. If incubation is performed in a water bath make sure that the collection microtubes are not fully submerged and that any remaining water is removed prior to removing the caps in step 5.

- 5. Carefully remove the caps, and add 200 pl ethanol (96-100%) to each sample.
- 6. Seal the collection microtubes properly using the caps provided. Place a clear cover over each rack of collection microtubes, and shake the racks vigorously up and down for 15 s. To collect any solution from the caps, contribute the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.
 Do not prolong this step.

IMPORTANT: The rack of collection microtubes must be vigorously shaken up and down with both hands to obtain a homogeneous lysate. Inverting the rack of collection microtubes is not sufficient for mixing. The genomic DNA will not be sheared by vigorous shaking. The lysate and ethanol should be mixed immediately and thoroughly to yield a homogeneous solution.

- 7. Place two Divisory 96 plates on top of S Blocks (provided). Mark the Divisory 96 plates for later sample identification.
- 8. Remove and discard the caps from the callection microtubes. Carefully transfer the lysis mixture (maximum 900 pt) of each sample from step 6 to each well of the DNieasy 96 plates.

Take care not to wet the rims of the wells to avoid aerosols during centrifugation. Do not transfer more than 900 pt per well.

Note: Lowering pipet tips to the bottoms of the wells may cause sample overflow and cross-contamination. Therefore, remove one set of caps at a time, and begin drawing up the samples as soon as the pipet tips contact the liquid. Repeat until all the samples have been transferred to the DiNeasy 96 plates.

9. Seal each Diseasy 96 plate with an AirPore Tape Sheet (provided). Centrifuge for 4 min at 6000 rpm.

AirPore Tape prevents cross-contamination between samples during centrifugation. After centrifugation, check that all of the lysate has passed through the membrane in each well of the DNeasy 96 plates. If lysate remains in any of the wells, centrifuge for a further 4 min.

10. Remove the tape. Carefully add 500 µl Buffer AW1 to each sample.

Note: Ensure that ethanol has been added to Buffer AW1 prior to use.

11. Seal each DNeasy 96 plate with a new AirPore Tape Sheet (provided). Centrifuge for 2 min at 6000 rpm.

12. Remove the tape. Carefully add 500 µl Buffer AW2 to each sample.

Note: Ensure that ethanol has been added to Buffer AW2 prior to use.

13. Centrifuge for 15 min at 6000 rpm.

Do not seal the plate with AirPore Tape.

The heat generated during centrifugation ensures evaporation of residual ethanol in the sample (from Buffer AW2) that might otherwise inhibit downstream reactions.

- Place each DNeasy 96 plate in the correct orientation on a new rack of Elution Microtubes RS (provided).
- 15. To eluie the DNA, add 200 pl Buffer AE to each sample, and seal the DNeasy 96 plates with new AirPore Tape Sheets (provided). Incubate for 1 min at room temperature (15-25°C). Centrifuge for 4 min at 6000 rpm.

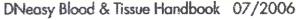
200 µl Buffer AE is sufficient to elute up to 75% of the DNA from each well of the DNeasy 96 plate.

Elution with volumes less than 200 µl significantly increases the final DNA concentration of the eluate but may reduce overall DNA yield. For samples containing less than 1 µg DNA, elution in 50 pl Buffer AE is recommended.

16. Recommended: For maximum DNA yield, repeat step 15 with another 200 µ! Buffer AE.

A second elution with 200 µl Buffer AF will increase the total DNA yield by up to 25%. However due to the increased volume, the DNA concentration is reduced. If a higher DNA concentration is desired, the second elution step can be performed using the 200 µl elucte from the first elution. This will increase the yield by up to 15%.

Use new caps (provided) to seal the Elution Microtubes RS for storage.



Protocol: Purification of Total DNA from Animal Tissues (DNeasy 96 Protocol)

This protocol is designed for high-throughput purification of total DNA from animal tissues, including rodent tails.

Important points before starting

- If using the DNeasy 96 Blood & Tissue Kit for the first time, read "Important Notes" (page 15).
- All centrifugation steps are carried out at room temperature (15-25°C).
- Optional: RNase A may be used to digest RNA during the procedure. RNase A is not provided in the DNeasy 96 Blood & Tissue Kit (see "Copuration of RNA", page 19).

Things to do before starting

- Buffer AL should be premixed with ethanol before use. Add 90 ml ethanol (96–100%) to the bottle containing 86 ml Buffer AL or 260 ml ethanol to the bottle containing 247 ml Buffer AL and shake thoroughly. Mark the bottle to indicate that ethanol has been added. (Please note that, for purification of DNA from animal blood, Buffer AL must be used without ethanol. Buffer AL can be purchased separately if the same kit will be used for purification of DNA from animal blood.)
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C for 5 min until the precipitates have fully dissolved.
- Mix Buffer AW1 before use by inverting several times.
- Preheat an incursator to 56°C for use in step 4.
- If using frozen tissue, equilibrate the sample to room temperature. Avoid repeated thawing and freezing of samples since this will lead to reduced DNA size.

Procedure

1. Cut up to 20 mg tissue (up to 10 mg spleen) into small pieces. For radent tails, place one (rat) or two (mouse) 0.4-0.6 cm lengths of tail into a collection microtube. Earmark the animal appropriately. Use a 96-Well-Plate Register (provided) to identify the position of each sample.

Ensure that the correct amount of starting material is used (see "Starting amounts of samples", page 15). For tissues such as spleen with a very high number of cells for a given mass of tissue, no more than 10 mg starting material should be used.

We strongly recommend to cut the tissue into small pieces to enable more efficient lysis. If desired, lysis time can be reduced by disrupting the sample using a bead mill, such as the QIAGEN Tissuelyser (see page 56 for ordering information), before addition of Buffer ATL and proteinase K. A supplementary protocol for simultaneous disruption of up to 48 tissue samples using the Tissuelyser can be obtained by contacting QIAGEN Technical Services (see back cover).

For rodent tails, a maximum of 1.2 cm (mouse) or 0.6 cm (rat) tail should be used. When purifying DNA from the tail of an adult mouse or rat, it is recommended to use only 0.4–0.6 cm.

Store the samples at -20°C until a suitable number has been collected (up to 192 samples). Samples can be stored at -20°C for several weeks to months without any reduction in DNA yield. DNA yields will be approximately 10–30 µg, depending on the type, length, age, and species of sample used (see "Expected yields", page 22).

Keep the clear covers from the collection microtube racks for use in step 3.

2. Prepare a proteinase K-Buffer ATL working solution containing 20 µl proteinase K stock solution and 180 µl Buffer ATL per sample, and mix by vortexing. For one set of 96 samples, use 2 ml proteinase K stock solution and 18 ml Buffer ATL. Immediately piper 200 µl working solution into each collection microtube containing the tail sections on tissue samples. Seal the microtubes properly using the caps provided.

Note: Check Buffer ATL for precipitate. If necessary, dissolve the precipitate by incubation at 56°C for 5 min before preparing the working solution.

IMPORTANT: After preparation, the proteinase K-Buffer ATL working solution should be dispensed immediately into the collection microtubes containing the tail or tissue samples. Incubation of the working solution in the absence of substrate for >30 min reduces tysis efficiency and DNA purity.

3. Ensure that the microtubes are properly sealed to avoid leakage during shaking. Place a clear cover (saved from step 1) over each rack of collection microtubes, and mix by inverting the rack of collection microtubes. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then step the centrifuge. It is essential that the samples are completely submerged in the proteinase K-Buffer ATL working solution after centrifugation.

If the proteinase K-Buffer ATL working solution does not completely cover the sample, increase the volume of the solution to 300 µl per sample (additional reagents are available separately; see page 56 for ordering information). Do not increase volumes above 300 µl as this will exceed the capacity of the collection microtubes in subsequent steps.

Keep the clear covers from the collection microtube racks for use in step 5.

4. Incubate at 56°C overnight or until the samples are completely lysed. Place a weight on top of the caps during the incubation. Mix accasionally during incubation to disperse the sample, or place on a racking platform.

Lysis time varies depending on the type, age, and amount of tail or tissue being processed. Lysis is usually complete in 1–3 h or, for rodent tails, 6–8 h, but optimal results will be achieved after overnight lysis.

After incubation the lysate may appear viscous, but should not be gelatinous as it may clog the DNeasy 96 membrane. If the lysate appears very gelatinous, see the "Troubleshooting Guide", page 47, for recommendations/

Note: Do not use a rotary- or vertical-type shaker as continuous rotation may release the caps. If incubation is performed in a water bath make sure that the collection microtubes are not fully submerged and that any remaining water is removed prior to centrifugation in step 5.

5. Ensure that the microtubes are properly sealed to avoid leakage during shaking. Place a clear cover over each rack of collection microtubes and shake the racks vigorously up and down for 15 s. to collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.

IMPORTANT: The rack of collection microtubes must be vigorously shaken up and down with both hands to obtain a homogeneous lysate. Inverting the rack of collection microtubes is not sufficient for mixing. The genomic DNA will not be sheared by vigorous shaking.

Keep the clear covers from the collection microtube racks for use in step 7.

Ensure that tysis is complete before proceeding to step 6. The lysate should be homogeneous following the vigorous shaking. To check this, slowly invert the rack of collection microtubes (making sure that the caps are tightly closed) and look for a gelatinous mass. If a gelatinous mass is visible, lysis needs to be extended by adding another 100 µl Buffer ATL and 15 µl proteinase K, and incubating for a further 3 h. It is very important to ensure that samples are completely lysed to achieve optimal yields and to avoid clogging of individual wells of the DNeasy 96 plate.

Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml). Close the collection microtubes with fresh caps, mix by shaking vigorously, and incubate for 5 min at room temperature. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge. Remove the caps, and continue with step 6.

Transcriptionally active tissues such as liver and kidney contain high levels of RNA, which will copurify with genomic DNA. For tissues that contain low levels of RNA, such as rodent tails, or if residual RNA is not a concern, RNase A digestion is usually not necessary.

6. Carefully remove the caps. Add 410 µl premixed Buffer At-ethanol to each sample.

Note: Ensure that ethanol has been added to Buffer AL prior to use (see "Buffer AL", page 18).

Note: A white precipitate may form upon addition of Buffer AL-ethanol to the lysate. It is important to apply all of the lysate, including the precipitate, to the DNeasy 96 plate in step 9. This precipitate does not interfere with the DNeasy procedure or with any subsequent application

If the volumes of Buffer ATL and proteings (K) were increased in steps 3 or 5, increase the volume of Buffer At and ethanol accordingly. For example, 300 µl proteinase K–Buffer ATL working solution will require 615 µl Buffer AL—ethanol.

7. Ensure that the microtubes are properly sealed to avoid leakage during shaking. Place a clear cover over each ruck of collection microtubes and shake the racks vigorously up and down for 15 s. To collect any solution from the caps, centrifuge the collection microtubes. Allow the centrifuge to reach 3000 rpm, and then stop the centrifuge.

Do not prolong this step.

IMPORTANT: The rack of collection microtubes must be vigorously shaken up and down with both hands to obtain a homogeneous lysate. Inverting the rack of collection microtubes is not sufficient for mixing. The genomic DNA will not be sheared by vigorous shaking. The lysate and Buffer AL—ethanol should be mixed immediately and thoroughly to yield a homogeneous solution.

- 8. Place two DNeasy 96 plates on top of S-Blocks (provided). Mark the DNeasy 96 plates for later sample identification.
- 9. Remove and discard the caps from the collection microtubes. Carefully transfer the lysate (maximum 900 µl) of each sample from step 7 to each well of the DNeasy 96 plates.

Take care not to wet the rims of the wells to avoid aerosols during centrifugation. Do not transfer more than 900 µl per well.

Note: Lowering pipet tips to the bottoms of the wells may cause sample overflow and cross-contamination. Therefore, remove one set of caps at a time, and begin drawing up the samples as soon as the pipet tips contact the liquid. Repeat until all the samples have been transferred to the DNeasy 96 plates.

Note: If the volume of proteinase K-Buffer ATL working solution was increased in steps 3 or 5, transfer no more than 900 µl of the supernatant from step 7 to the DNeasy 96 plate. Larger amounts will exceed the volume capacity of the individual wells. Discard any remaining supernatant from step 7 as this will not contribute significantly to the total DNA yield.

10. Seal each DNeasy 96 plate with an AirPore Tape Sheet (provided). Centrifuge for 10 min at 6000 rpm.

AirPore Tape prevents cross-contamination between samples during centrifugation.

After centrifugation, check that all of the lysate has passed through the membrane in each well of the DNeasy 96 plates. If lysate remains in any of the wells, centrifuge for a further 10 min.

11. Remove the tape. Carefully add 500 pl Buffer AV/1 pb/each sample.

Note: Ensure that ethanol has been added to Buffer AVVI prior to use.

It is not necessary to increase the volume of Buller AW1 if the volume of proteinase K-Buffer ATL working solution was increased in steps 3 or 5.

- 12. Seal each DNeasy 96 plate with a new AirPore Tape Sheet (provided). Centrifuge for 5 min at 6000 rpm.
- 13. Remove the tape. Carefully and 500 pt Buffer AW2 to each sample.

More: Ensure that ethanol has been added to Buffer AW2 prior to use.

It is not necessary to increase the volume of Buffer AW2 if the volume of proteinase K-Buffer ATL working solution was increased in steps 3 or 5.

14. Centrifuge for 15 pair at 6000 ppm.

Do not seal the plate with AirPore Tape.

The heat generated during centrifugation ensures evaporation of residual ethanol in the sample (from Buffer AW2) that might otherwise inhibit downstream reactions.

- 15. Place each Diversy 96 plate in the correct orientation on a new rack of Elution Microtubes RS (provided).
- 16. To elute the DNA, add 200 µl Buffer AE to each sample, and seal the DNeasy 96 plates with new AirPare Tape Sheets (provided). Incubate for 1 min at room temperature (15–25°C). Centrifuge for 2 min at 6000 rpm.

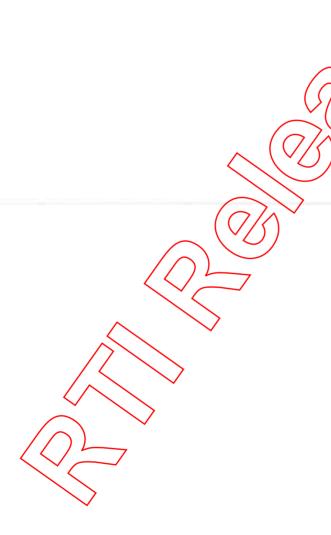
200 μ l Buffer AE is sufficient to elute up to 75% of the DNA from each well of the DNeasy 96 plate.

Elution with volumes less than 200 µl significantly increases the final DNA concentration of the eluate but may reduce overall DNA yield. For samples containing less than 1 µg DNA, elution in 50 µl Buffer AE is recommended.

 Recommended: For maximum DNA yield, repeat step 16 with another 200 µft furfer AE.

A second elution with 200 μ l Buffer AE will increase the total DNA yield by up to 25%. However due to the increased volume, the DNA concentration is reduced. If a higher DNA concentration is desired, the second elution step can be performed using the 200 μ l eluate from the first elution. This will increase the yield by up to 15%.

Use new caps (provided) to seal the Elution Microtubes RS for storage.



Protocol: Pretreatment for Paraffin-Embedded Tissue

This protocol is designed for purification of total DNA from fixed, paraffin-embedded tissues using the DNeasy Blood & Tissue Kit. The protocol describes the preliminary removal of paraffin by extraction with xylene.

Important points before starting

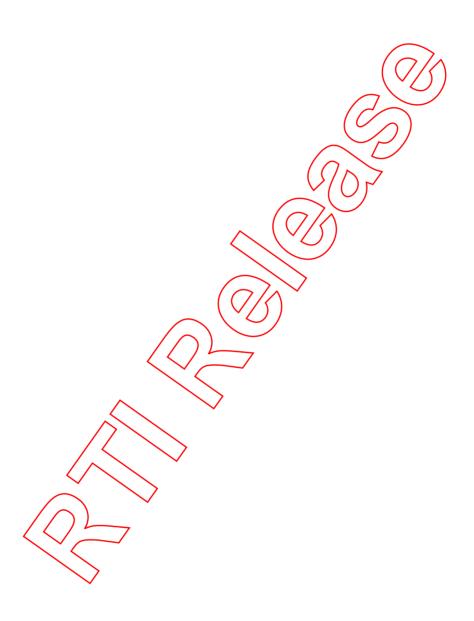
- The length of DNA purified from fixed tissues is usually <650 bp, depending on the type and age of the sample and the quality of the fixative used.
- Use of fixatives such as alcohol and formalin is recommended. Fixatives that cause cross-linking, such as osmic acid, are not recommended as it can be difficult to obtain amplifiable DNA from tissue fixed with these agents.
- Lysis time will vary from sample to sample depending on the type of tissue processed.
- Yields will depend both on the size and the age of the sample processed. Reduced yields compared with fresh or frozen tissues are to be expected. Therefore, eluting purified DNA in 50–100 µl Buffer AE is recommended.
- This pretreatment protocol has not been thoroughly tested and optimized for high-throughput DNA purification using the Diveasy 96 Blood & Tissue Kit. As a general guideline, we recommend to decrease the amount of starting material when using this protocol with the DNeasy 96 Blood & Tissue Kit.

Things to do before starting

Preheat a heating block, incubator, or water bath to 37°C for use in step 9.

- 1. Place a small section (not more than 25 mg) of paraffin-embedded tissue in a 2 ml microcentrifuge tabe (not provided).
- 2. Add 1200 playiene. Vortex vigorously.
- 3. Centrifuge in a microgentrifuge of full speed for 5 min at room temperature (15-25°C).
- 4. Remove supernations by pipetting. Do not remove any of the pellet.
- 5. Add 1200 µl ethanol (96-100%) to the pallet to remove residual xylene, and mix gently by vortexing.
- 6. Centrifuge in a microcentrifuge at full speed for 5 min at room temperature.
- 7. Carefully remove the ethanol by pipetting. Do not remove any of the pellet.
- 8. Repeat steps 5-7 once.

- Incubate the open microcentrifuge tube at 37°C for 10-15 min until the ethanol has evaporated.
- 10. Resuspend the tissue pellet in 180 µl Buffer ATL, and continue with step 2 of the protocol "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)", page 29.



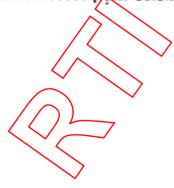
Protocol: Pretreatment for Formalin-Fixed Tissue

This protocol is designed for purification of total DNA from fixed, paraffin-embedded tissues. The protocol describes the preliminary washing with PBS to remove the fixative.

important points before starting

- The length of DNA purified from fixed tissues is usually <650 bp, depending on the type and age of the sample and the quality of the fixative used.
- Use of fixatives such as alcohol and formalin is recommended. Fixatives that cause cross-linking, such as osmic acid, are not recommended as it can be difficult to obtain amplifiable DNA from tissue fixed with these agents
- Lysis time will vary from sample to sample depending on the type of tissue processed.
- Yields will depend both on the size and the age of the sample processed. Reduced yields compared with fresh or frozen tissues are to be expected. Therefore, eluting purified DNA in a total volume of 50–100 µl Buffer AE is recommended.
- This pretreatment protocol has not been thoroughly lested and optimized for high-throughput DNA purification using the DNedsy 95 Blood & Tissue Kit. As a general guideline, we recommend to decrease the amount of starting material when using this protocol with the DNedsy 96 Blood & Tissue Kit.

- 1. Wash the sample (not more than 25 mg) twice in PBS to remove the fixative.
- 2. Discard the PBS and continue with step 1 of the protocol "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)", page 28.



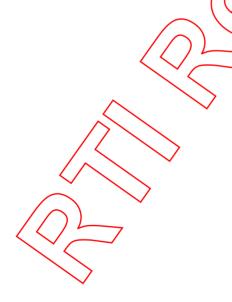
Protocol: Pretreatment for Gram-Negative Bacteria

This protocol is designed for purification of total DNA from Gram-negative bacteria, such as *E. coli*. The protocol describes the preliminary harvesting of bacteria before DNA purification.

Important points before starting

- See "Quantification of starting material", page 17, for details of how to collect and store samples, and how to determine the number of cells in a bacterial culture.
- This pretreatment protocol has not been thoroughly tested and optimized for high-throughput DNA purification using the DNeasy 96 Blood & Tissue Kit. As a general guideline, we recommend to decrease the amount of starting material when using this protocol with the DNeasy 96 Blood & Tissue Kit.

- 1. Horvest cells (maximum $2 \times 10^\circ$ cells) in a mixture outsituge tube by centrifuging for 10 min of 5000 x g (7500 rpm). Discord supernesent.
- 2. Resuspend pellet in 180 pl Buffer ATL.
- 3. Continue with step 2 of the protocol "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)", page 29.



Protocol: Pretreatment for Gram-Positive Bacteria

This protocol is designed for purification of total DNA from Gram-positive bacteria, such as Corynebacterium spp. and B. subtilis. The protocol describes the preliminary harvesting of bacteria and incubation with lysozyme to lyse their cell walls before DNA purification.

Important points before starting

- See "Quantification of starting material", page 17, for details of how to collect and store samples, and how to determine the number of cells in a bacterial culture.
- Ensure that ethanol has not been added to Buffer AL (see "Buffer AL", page 18).

 Buffer AL can be purchased separately (see page 56 for ordering information).
- This pretreatment protocol has not been thoroughly tested and optimized for high-throughput DNA purification using the DNeasy 96 Blood & Tissue Kit. As a general guideline, we recommend to decrease the amount of starting material when using this protocol with the DNeasy 96 Blood & Tissue Kit)

Things to do before starting

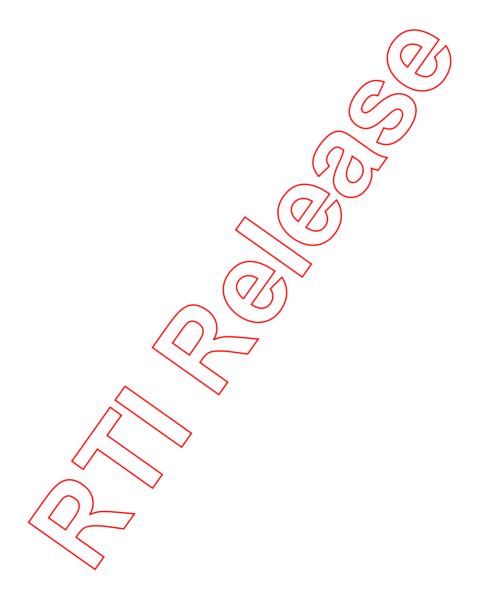
- Prepare enzymatic lysis buffer as described in Equipment and Reagents to Be Supplied by User", page 14.
- Preheat a heating block or water both to 37°C for use in step 3.

- 1. Hervest cells (maximum $2 \times 10^\circ$ cells) in a microcentrifuge tube by centrifuging for 10 min at 5000 \times g (7500 rpm). Discord supernatant.
- 2. Resuspend bacterior pellet in 180 pl enzymatic lysis buffer.
- 3. Incubate for at least 30 min at 37°C.
 - After incubation, heat the heating block or water bath to 56°C if it is to be used for the incubation in step 5.
- 4. Add 25 pt proteinase K and 200 pt Buffer AL (without ethanol). Mix by vortexing. Note: Do not add proteinase K directly to Buffer AL.
 - Ensure that ethanol has not been added to Buffer AL (see "Buffer AL", page 18). Buffer AL can be purchased separately (see page 56 for ordering information).
- 5. Incubate at 56°C for 30 min.
 - Optional: If required, incubate at 95°C for 15 min to inactivate pathogens. Note that incubation at 95°C can lead to some DNA degradation.

6. Add 200 pl ethanol (96-100%) to the sample, and mix thoroughly by vortexing.
It is important that the sample and the ethanol are mixed thoroughly to yield a homogeneous solution.

A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the DNeasy Mini spin column. This precipitate does not interfere with the DNeasy procedure.

7. Continue with step 4 of the protocol "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)", page 30.



Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or molecular biology applications (see back cover for contact information).

Comments and suggestions

Low yield

- a) Storage of starting material
- b) Too much starting material
- Insufficient mixing of sample with Buffer AL and ethanol before binding

d) DNA inefficiently eluted

e) Buffer AW1 or Buffer AW2 prepared incorrectly

DNA yield is dependent on the type, size, age, and storage of starting material. Lower yields will be obtained from material that has been inappropriately stored (see "Sample collection and storage", page 17). In future preparations, reduce the amount of starting material used (see "Quantification of starting material", page 16).

preparations mix sample first with Buffer AL and then with ethanol by pulse vortexing for 15's each time before applying the sample to the Diverse Mini spin column.

Diversy % protocols: In future preparations, ensure that samples are mixed by vigorous shaking, as described in the protocols, before applying the sample to the DNeasy 96 plate.

Increase elution volume to 200 µl and perform another elution step. See also "Elution of pure nucleic acids", page 21. Check that ethanol was added before applying the sample to the DNeasy Mini spin column. Check that any precipitate in Buffer ATL and/or Buffer AL was dissolved before use.

Make sure that ethanol has been added to Buffer AW1 and Buffer AW2 before use (see "Things to do before starting", pages 25, 28, 31, and 35).

- Water used instead of Buffer AE for elution
- g) Animal rissue: Insufficient lysis

The low pH of deionized water from some water purifiers may reduce DNA yield. When eluting with water, ensure that the pH of the water is at least 7.0.

In future preparations, reduce the amount of starting material used (see "Quantification of starting material", page 17).

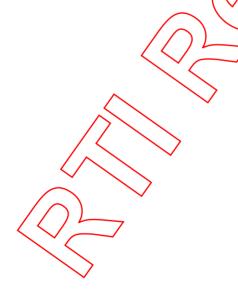
Cut tissue into smaller pieces to facilitate lysis. After lysis, vortex sample vigorously; this will not damage or reduce the size of the DNA.

If a substantial gelatinous pellet remains after incubation and vortexing, extend incubation time at 56°C for proteinase K digest and/or increase amount of proteinase K to 40 µl. (For DNeasy 96 proteools,) always check that the sample is completely lysed before addition of Buffer AL and ethanol. If a gelatinous mass is still present after the overnight incubation, lysis needs to be extended.)

Ensure that the sample is fully submerged in the buffer containing proteinase K. If necessary, double the amount of Buffer ATL and proteinase K, and use a 2 ml microcentrifuge tube for lysis. Remember to adjust the amount of Buffer AL and ethanol proportionately in subsequent steps. (For example, a lysis step with 360 µl Buffer ATL plus 40 µl proteinase K will require 400 µl Buffer AL plus 400 µl ethanol to bind DNA to the DNeasy membrane).

DNeasy spin-column protocols: Pipet the sample into the DNeasy Mini spin column in two sequential loading steps. Discard flow-through between these loading steps.

Diverse 96 protocols: Transfer a maximum of 900 µl of each sample to the Diverse 96 plate.



h) Bacteria: Insufficient lysis In future preparations, extend incubation cell-wall-lysing enzyme increase amount of lysing enzyme.

Harvest bacteria during early log phase of growth (see "Sample collection and storage", page 15).

1 DNeasy spin-column protocols: DNA not bound to DNeasy Mini spin column

Check that ethanol was added before applying the sample to the DNeasy Mini spin column.

DNessy 96 protectors: Inefficient i) DNA elution

Repeat elution with Buffer AE preheated to 70°C.

After addition of Duffer AE preheated to 70°C, the DNeasy 96 plate should be incubated of room temperature for 1 min. To increase electron efficiency, extend the incubation to 5 min at 70°C.

Diversy 96 protocols: Unequal k) volumes of Buffer AE or water delivered by the multichannel pipet

Ensure that all tips are firmly fitted to the pipst. Check liquid levels in tips before dispensing.

Diversy Mini spin column or Diversy 96 plate clarged

Too much starting material and/or insufficient lysis

Increase aforce and/or duration of ceptrifugation step. In future preparations, reduce the amount of starting material used (see "Quantification of starting material", page 17). For rodent tails or bacteria, see also "insufficient lysis" in the "Low yield" section above.

Low concentration of DNA in the elucite Second elation step diluted the DNA

Use a new collection tube for the second eluate to prevent dilution of the first eluate. Reduce elution volume to 50–100 pl. See "Elution of pure nucleic acids", page 21.

A260/A280 ratio of purified DNA is low

Use 10 mM Tris-Cl, pH 7.5 instead of water to dilute the sample before measuring purity. See "Appendix A: Determination of Yield,

Purity, and Length of DNA", page 52.

a) Water used instead of buffer to measure A_{260}/A_{280}

See "Low yield", above.

b) Inefficient cell lysis

A260/A280 ratio of purified DNA is high

High level of residual RINA

Perform the optional RNase treatment in the protocol.

DNA does not perform well in downstream applications

a) Salt carryover

Ensure that Buffer AW2 has been used at room temperature (15-25°C).

Ensure that Buffer AW1 and Buffer AW2 were added in the correct order.

b) Ethanol carryover

when washing with Buffer AW2, the column is centrifuged for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Following the centrifugation step, remove the DNeasy Mini spin column carefully so that the column does not come into contact with the flow-through. If ethanol is visible in the DNeasy Mini spin column (as either drops or a film), discard the flow-through, keep the collection tube, and centrifuge for a further 1 min at 20,000 x g.

PNeasy 96 protocols: Incubate the DNeasy 96 plate, uncovered, in an oven or incubator for 10 min at 80°C after the second wash to remove all traces of Buffer AW2.

For PCR applications, a single-copy gene can typically be detected after 35 PCR cycles with 100 ng template DNA.

Sample repeatedly frozen and thawed

Avoid repeated freezing and thawing of starting material.

Old samples often yield only degraded DNA.

White precipitate in Buffer ATL or Buffer AL

Sample too old

Too much DNA used

White precipitate may form at low temperature after prolonged storage

Any precipitate formed when Buffer ATL or Buffer AL are added must be dissolved by incubating the buffer at 56°C until it disappears.

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c)

b)

DNA sheared

Discolored membrane after wash with Buffer AW2, or colored cludie

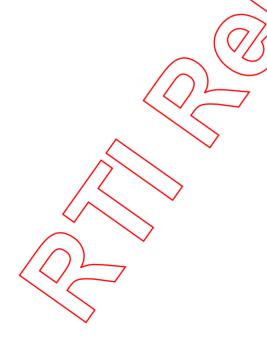
 a) Rodent wils: Hair not removed from rodent tails during preparation

DNeasy spin-column protocols: In future preparations, centrifuge lysate for 5 min at $20,000 \times g$ after digestion with proteinase K. Transfer supernatant into a new tube before proceeding with step 3.

Difference of protocols: In future preparations, centrifuge the rack of collection microtubes containing the lysates for 5 min at 6000 rpm at step 5. Remove the cops. Carefully transfer the lysates, without disturbing the pelleted debris, to emother rack of collection microtubes. Continue the protocol at step 6.

Reduce amount of blood used and/or double the amount of proteinase K used per preparation. Its using buffy coat instead of whole blood.

b) Animal bleed: Contamination with hemoglobin



Appendix A: Determination of Yield, Purity, and Length of DNA

Determination of yield and purity

DNA yield is determined by measuring the concentration of DNA in the eluate by its absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. Measure the absorbance at 260 nm or scan absorbance from 220–330 nm (a scan will show if there are other factors affecting absorbance at 260 nm; for instance, absorbance at 325 nm would indicate contamination by particulate matter or a dirty cuvette). An A_{260} value of 1 (with a 1 cm detection path) corresponds to 50 μ g DNA per milliliter water. Water should be used as diluent when measuring DNA concentration since the relationship between absorbance and concentration is based on extinction coefficients calculated for nucleic acids in water.* Both DNA and RNA are measured with a spectrophotometer at 260 nm; to measure only DNA in a mixture of DNA and RNA, a fluorimeter must be used.

An example of the calculations involved in DNA quantification is shown below.

Volume of DNA sample = 10

Dilution = 20 µl at DNA sample + 180 µl distilled water

(1) 10 dilution)

Measure absorbance of diluted sample in a 0.2 ml cuvette

 A_{260}

Concentration of DNA/sample = $50 \mu g/ml \times A_{260} \times dilution factor$

= 50 µg/ml x 0.2 x 10

 $= 100 \, \mu g/ml$

Total amount = concentration x volume of sample in milliliters

 $= 100 \, \mu g/ml \times 0.1 \, ml$

= 10 µg DNA

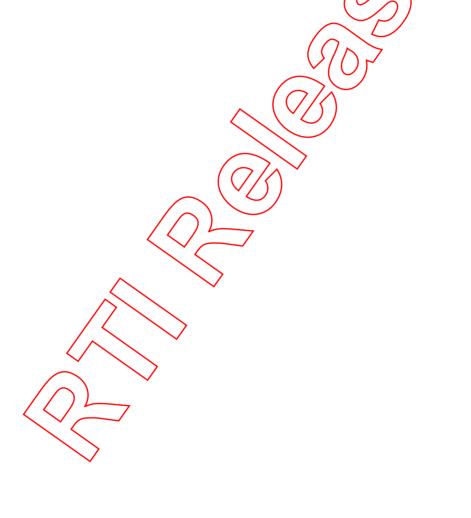
The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of DNA with respect to contaminants that absorb UV, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination. For accurate values, we recommend measuring absorbance in 10 mM Tris-Cl, pH 7.5, in which pure DNA has an A_{260}/A_{280} ratio of 1.8–2.0. Always be sure to calibrate the spectrophotometer with the same solution.

Wilfinger, W.W., Mackey, M., and Chomcynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques 22, 474.

Determination of length

The precise length of genomic DNA should be determined by pulse-field gel electrophoresis (PFGE) through an agarose gel. To prepare the sample for PFGE, the DNA should be concentrated by alcohol precipitation and the DNA pellet dried briefly at room temperature (15–25°C) for 5–10 minutes. Avoid drying the DNA pellet for more than 10 minutes since overdried genomic DNA is very difficult to redissolve. Redissolve in approximately 30 µl TE buffer, pH 8.0,* for at least 30 minutes at 60°C. Load 3–5 µg of DNA per well. Standard PFGE conditions are as follows:

- 1% agarose gel in 0.5 x TBE electrophoresis buffer*
- switch intervals = 5-40 seconds
- run time = 17 hours
- voltage = 170 V

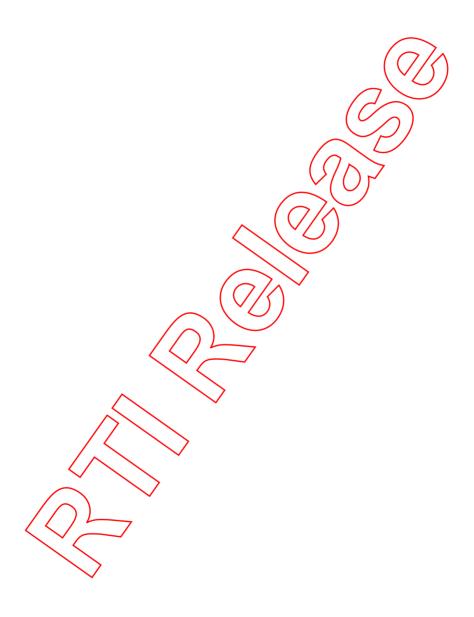


^{*} When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Appendix B: Cleaning S-Blocks

Cleaning 5-Blocks

To avoid cross-contamination, after each use rinse the S-Blocks thoroughly in tap water, incubate for 1 min at room temperature in 0.4 M HCl,* empty, and wash thoroughly with distilled water. Used S-Blocks can also be autoclaved after washing. Additional S-Blocks can be ordered separately (see page 55 for ordering information).



^{*} When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Product	Contents	Cat. no.
DNeasy Blood & Tissue Kit (50)	50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504
DNeasy Blood & Tissue Kit (250)	250 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69506
DNeasy 96 Blood & Tissue Kit (4)*	For 4 x 96 DNA minipreps: 4 DNeasy 96 Plates, Proteinuse K, Buffers, S-Blocks, AirPore Tope Sheets, Collection Microtubes (1.2 ml), Elution Microtubes R3 Caps, 96-Well Plate Registers	69581
DNeasy 96 Tissue Kit (12)*	For 12 x 96 DNA ministers: 12 DNeasy 96 Plates, Proteinase K, Buffers, & Blocks, AirPore Tape Sheets, Collection Microtubes (1.2 ml), Elution Microtubes RS, Caps, 96 Mell Plate Registers	69582
GIAGEN 96-Well Plate Centrifuga	Kon System	
Centrifuge 4-15C	Upiversal Joboratory centrifuge with brushless motor	Inquire
Centrifuge 4K15C	Universal refrigerated laboratory centrifuge with brushless motor	Inquire
Plate Rotor 2 x 96	Rotor for 2 QIAGEN 96-well plates, for use with QIAGEN Centrifuges	81031
Accessories		
Collection Tubes (2 mil)	1000 Collection Tubes (2 ml)	19201
Collection Microtobes (racked, 10 x 96)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560
Collection Microtube Caps (120 x 8)	Nonsterile polypropylene caps for collection microtubes (1.2 ml) and round-well blocks, 960 in strips of 8	19566
S-Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19585

^{*} Larger kit sizes and/or formats available; please inquire.

roduci	Contents	Cat. no.
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks: 50 sheets per pack	19571
TissueRuptor	Handheld rotor-stator homogenizer	Inquire
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor	990890
Tissuelyser	Universal laboratory mixer-mill disruptor	Inquire
Tissuelyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2.0 m/microcentrifuge tubes on the Tissuelyser	69982
TissueLyser Adapter Set 2 x 96	2 sets of Adapter Plates for use with Collection Microtubes (racked) on the Tissuetyser	69984
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TiesueLyser system	69989
QIAGEN Proteinase K (2 ml)	2 m [>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10 ml)	10 m/ 1>00 mAU/ml, solution)	19133
RNase A (17,500 U)	2.5 ml (100 mg/ml; 7000 units/ml, solution)	19101
Buffer AL (216 ml)	216 ml Lysis Buffer	19075
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer for 1000 preps	19076
Buffer AW1 (Zonsentrate, 242 ml)	242 ml Wash Buffer (1) Concentrate	19081
Buffer AW2 (Concentrate, 324 ml)	324 ml Wash Buffer (2) Concentrate	19072
Buffer AE (240 ml)	240 ml Elution Buffer	19077
elated products		
QIAGEN Genomic-tip 20/G	25 columns	10223
QIAGEN Genomic-tip 100/G	25 columns	10243
QIAGEN Genomic-tip 500/G	10 columns	10262
Blood & Cell Culture DNA Mini Kit (25)	25 QIAGEN Genomic-tip 20/G, QIAGEN Protease, Buffers	13323

roduct	Contents	Cat. no.
Blood & Cell Culture DNA Midi Kit (25)	25 QIAGEN Genomic-tip 100/G, QIAGEN Protease, Buffers	13343
Blood & Cell Culture DNA Maxi Kit (10)	10 QIAGEN Genomic-tip 500/G, QIAGEN Protease, Buffers	13362
BioSprint 15 DNA Blood Kit (45)*	For 45 preps on the BioSprint 15 workstation: 5-Rod Covers, 5-Tube Strips, MagAttract Suspension G, Buffers and Reagents	940014
BioSprint 96 DNA Blood Kit (48)*	For 48 preps on the BioSprint 26 workstation: Large 96-Rod Covers, 96-Well Microplates MR, 5-Blocks, MagAttract Suspension G, Buffers and Reagents	940054
RNeasy® Mini Kit (50)*	For 50 RNA minipreps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104
RNeasy Midi Kit (10)*	For 10 RNA midipreps: 10 RNeasy Midi Spin Columns, Collection Tubes (15/ml), RNase-free Reagents and Buffers	75142
RNeasy Maxi Kit (12)	For 12 RNA maxipreps: 12 RNeasy Maxi Spin Columns, Collection Tubes (50 ml), RNase-free Reagents and Buffers	75162
RNeasy Protect Mini Kit (50)	For RNA stabilization and 50 RNA minipreps: RNAlater® RNA Stabilization Reagent (50 ml), 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74124

^{*} Larger kit sizes and/or formats available; please inquire.

Product	Contents	Cet. no.
RNeasy Fibrous Tissue Mini Kit (50)*	For 50 RNA minipreps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), Proteinase K, RNase-free DNase I, RNase-free Reagents and Buffers	74704
RNeasy Lipid Tissue Mini Kit (50)*	For 50 RNA minipreps: 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), QIAzoltysis Reagent, RNase-free Reagents and Buffers	74804

^{*} Larger kit sizes and/or formats available; please inquire.

QIAGEN Distributors and Importers

Please see the back cover for contact information for your local QIAGEN office.

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Bench Protocol: Animal Blood (Spin-Column Protocol)



Note: Before using this bench protocol, you should be completely familiar with the safety information and detailed protocols in the DNeasy Blood & Tissue Handbook.

important points before starting

- Perform all centrifugation steps at room temperature (15-25°C).
- If necessary, redissolve any precipitates in Buffer AL.
- Ensure that ethanol has been added to Buffers AW1 and AW2.
- Preheat a thermomixer, shaking water both, or rocking platform for heating at 56°C.

- 1a. Nonnucleated blood: Pipet 20 pl proteinate Oipto a 1.5 ml or 2 ml microcentrifuge tube. Add 50-100 pl anticoagulated blood. Adjust the volume to 220 pl with PBS.
- 1b. Nucleated blood: Pipet 20 µl proteinase Winto a 1.5 ml or 2 ml microcentrifuge tube. Add 5-10 µl anticoagulated blood. Adjust the volume to 220 µl with PBS.
- 1c. Cultured cells: Centrifuge maximum 5 x 10° cells for 5 min at 300 x g. Resuspend in 200 µl PBS. Add 20 µl profeinase K.
- 2. Add 200 pl Buffer AL. Alix by Voctoring. Incubate at 56°C for 10 min.
- 3. Add 200 µl ethanol (96-) 00%). Mix thoroughly by vortexing.
- Pipet the mixture into a Diversy Mini spin column in a 2 ml collection tube.
 Centrifuge at ≥6000 x g (\$000 rpm) for 1 min. Discard flow-through and collection tube.
- 5. Place the spin column in a new 2 ml collection tube. Add 500 µl Buffer AW1. Centrifuge for 1 min at ≥6000 x g. Discard flow-through and collection tube.
- 6. Place the spin column in a new 2 ml collection tube, add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x g (14,000 rpm). Discard flow-through and collection tube.
 - Remove the spin column carefully so that it does not come into contact with the flow-through.
- 7. Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube, and add 200 μ l Buffer AE for elution. Incubate for 1 min at room temperature. Centrifuge for 1 min at \geq 6000 \times g.
 - Recommended: Repeat this step for maximum yield.

Bench Protocol: Animal Tissues (Spin-Column Protocol)



Note: Before using this bench protocol, you should be completely familiar with the safety information and detailed protocols in the DNeasy Blood & Tissue Handbook.

Important points before starting

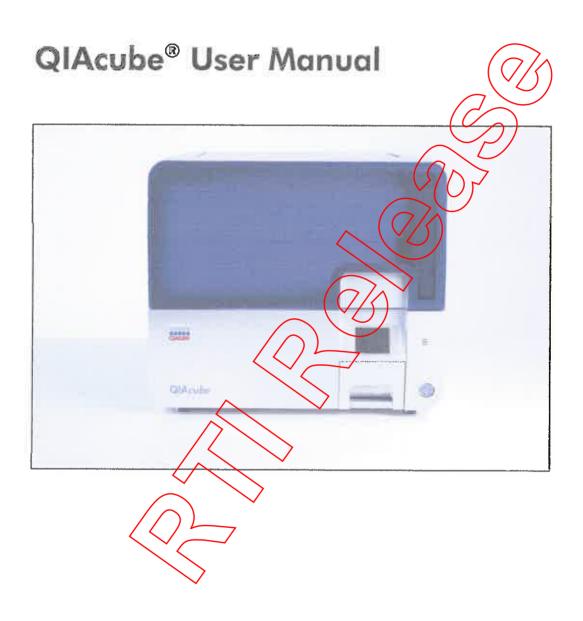
- Perform all centrifugation steps at room temperature (15-25°C).
- If necessary, redissolve any precipitates in Buffers ATL and AL.
- Ensure that ethanol has been added to Buffers AW1 and AW2.
- Preheat a thermomixer, shaking water bath, or rocking platform for heating at 56°C.
- If using frozen tissue, equilibrate the sample to room temperature.

Procedure

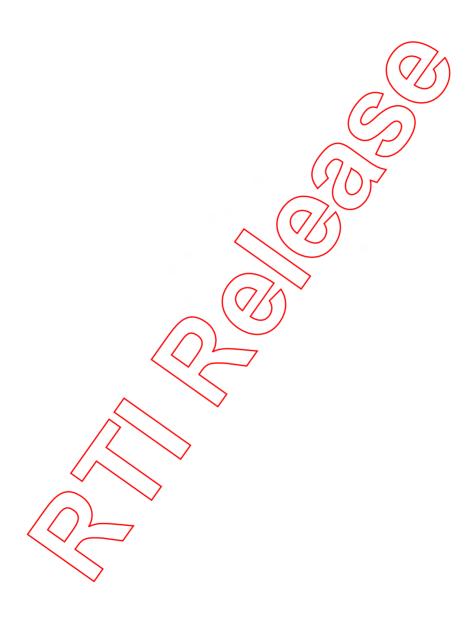
- 1. Cut tissue (up to 25 mg; up to 10 mg spleen) into small pieces, and place in 1.5 ml microcentrifuge tube. For radent tails, use one trail or two (mouse) 0.4–0.6 cm lengths of tail. Add 180 ul Buffer ATL.
- 2. Add 20 µl proteinase K. Mix by vortexing, and incubate at 56°C until completely lysed. Vortex accessionally during incubation, or place in a thermomixer, in a shaking water bath, or on a racking platform.

Lysis is usually complete in 1–3 hon for rodent tails, 6–8 h. Samples can be lysed overnight.

- 3. Vortex for 15 s. Add 200 pl Buffer AL to the sample. Mix thoroughly by vortexing. Then add 200 pl ethanel (96–100%). Mix again thoroughly.
 - Alternatively, premix Buffer AL and ethanol, and add together.
- 4. Piper the mixture into a QNeasy Mini spin column in a 2 ml collection tube. Centrifuge at \geq 6000 x g (8000 rpm) for 1 min. Discard flow-through and collection tube.
- 5. Place the spin column in a new 2 ml collection tube. Add 500 µl Buffer AW1. Centrifuge for 1 min at ≥6000 x g. Discard flow-through and collection tube.
- 6. Place the spin column in a new 2 ml collection tube. Add 500 μ l Buffer AW2. Centrifuge for 3 min at 20,000 x g (14,000 rpm). Discard flow-through and collection tube.
 - Remove the spin column carefully so that it does not come into contact with the flow-through.
- 7. Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube, and add 200 μ l Buffer A5 for elution. Incubate for 1 min at room temperature. Centrifuge for 1 min at \geq 6000 \times g.
 - Recommended: Repeat this step for maximum yield.







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1 Safety Information

Before using the QIAcube, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

The following types of safety information appear throughout this manual.

WARNING



The term WARNING is used to inform you about situations that could result in personal injury to you or other persons.

Details about these circumstances are given in a box like this one.

CAUTION



The term CAUTION is used to inform you about situations that could result in doctrage to the instrument or other equipment.

Details about these circumstances are given in a box like this one

The advice given in this manual is intended to supplement, not supersede, the normal safety requirements prevailing in the user's country.

1.1 Proper use

WARNING



Risk of personal injury and material damage [W1] Improper use of the QIAcube may cause personal injuries or damage to the instrument.

The QIAcube must only be operated by qualified personnel who have been appropriately trained.

Servicing of the QIAcube must only be performed by QIAGEN Field Service Specialists.

Perform the maintenance as described in Section 6. QIAGEN charges for repairs that are required due to incorrect maintenance.

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WARNING



Risk of personal injury and material damage [w2] The QlAcube is too heavy to be lifted by one person. To avoid personal injury or damage to the instrument, do not lift the instrument alone.

WARNING



Risk of personal injury and material damage [w3]
Do not attempt to move the QIAcube during operation.

CAUTION

Damage to the instrument

[C1]



Avoid spilling water or chemicals onto the alacube.

Damage caused by water or chemical spillage will void your warranty.

In case of emergency, switch off the Clacube at the power switch at the front of the instrument and unplug the power cord from the power outlet

CAUTION



Damage to the instrument

IC21

Only use QIAGEN® spin columns and QIAGEN Spin Kits with the QIAcube. Damage coused by use of other types of spin column or chemistries will void your warranty.

1.2 Electrical safety

Disconnect the line power cord from the power outlet before servicing.



WARNING

4

Electrical hazard

[W4]

Any interruption of the protective conductor (earth/ground lead) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous.

Intentional interruption is prohibited.

Lethal voltages inside the instrument

When the instrument is connected to line power, terminals may be live, and opening covers or removing parts is likely to expose live parts.

To ensure satisfactory and safe operation of the QIAcube, follow the advice below:

- The line power cord must be connected to a line power outlet that has a protective conductor (earth/ground).
- Do not adjust or replace internal parts of the instrument.
- Do not operate the instrument with any covers or parts removed.
- If liquid has spilled inside the instrument, switch off the instrument disconnect it from the power outlet, and contact QIAGEN Technical Services.

If the instrument becomes electrically unsafe, prevent other personnel from operating it, and contact QIAGEN Technical Services; the instrument may be electrically unsafe when:

- It or the line power cord appears to be damaged.
 - It has been stored under unfavorable conditions for a prolonged period.
- has been subjected to severe transport stresses.

1.3

Environment

Operating conditions

Warning



Explosive atmosphere

The QIAcube is not designed for use in an explosive atmosphere.

[W5]

QlAcube User Manual 06/2008

WARNING

Risk of explosion

[W6]



The QIAcube is intended for use with reagents and substances supplied with QIAGEN kits. Use of other reagents and substances may lead to fire or explosion.

CAUTION

Damage to the instrument

[C3]



Direct sunlight may bleach parts of the instrument and cause damage to plastic parts.

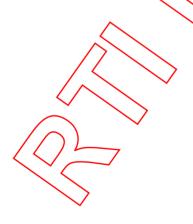
The QIAcube must be located out of direct sunlight

1.4 Biological safety

Specimens and reagents containing materials from humans should be treated as potentially infectious. Use safe laboratory procedures as outlined in publications such as Biosafety in Microbiological and Biomedical Laboratories, HHS (www.cdc.gov/od/obs/biostry/biosfty.htm).

Samples

Samples may contain infectious agents. You should be aware of the health hazard presented by such agents and should use, store, and dispose of such samples according to the required safety regulations.



WARNING



Samples containing infectious agents

IW71

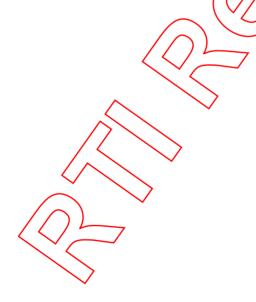
Some samples used with this instrument may contain infectious agents. Handle such samples with the greatest of care and in accordance with the required safety regulations.

Always wear safety glasses, 2 pairs of gloves, and a lab coat.

The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe, and that the instrument operators are suitably trained and not exposed to hazardous levels of infectious agents as defined in the applicable Material Safety Data Sheets (MSDSs) or OSHA,* ACGIH,† or COSHH‡ documents.

Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and lows.

- * OSHA: Occupational Safety and Health Administration (United States of America).
- [†] ACGIH: American Conference of Government Industrial Hygienists (United States of America).
- [‡] COSHH: Control of Substances Hazardous to Health (United Kingdom).



1.5 Chemicals

WARNING

Hazardous chemicals

IW81



Some chemicals used with this instrument may be hazardous or may become hazardous after completion of the protocol run.

Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Material Safety Data Sheets (MSDSs) or OSHA,* ACGIH,† or COSHH‡ documents.

Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

- * OSHA: Occupational Safety and Health Administration (United States of America).
- † ACGIH: American Conference of Government Industrial Hygienists (United States of America).
- † COSHH: Control of Substances Hazardous to Health (United Kingdom).

WARNING

Risk of fire

[W9]



When cleaning the Clacube with alcohol-based disinfectant, leave the Clacube door open to allow flammable vapors to disperse.

Only clean the QIAcube when worktable components have cooled down.

Toxic fomes

If working with volatile solvents or toxic substances, you must provide an efficient laboratory ventilation system to remove vapors that may be produced.

WARNING

Toxic fumes

IW101



Do not use bleach to disinfect used labware. Bleach in contact with salts from the buffers used can produce toxic fumes.

1.6 Waste disposal

Used labware, such as sample tubes, QIAGEN spin columns, filter-tips, or rotor adapters, may contain hazardous chemicals or infectious agents from the purification process. Such wastes must be collected and disposed of properly according to local safety regulations.

For information about how to dispose of the QIAcube, see Appendix A.

1.7 Mechanical hazards

The door of the QIAcube must remain closed during operation of the instrument. Only open the door when instructed to do so by the software.

WARNING

Moving parts

[W11]



To avoid contact with moving parts during operation of the QIAcube, the instrument must be operated with the door closed.

If the door sensor is not functioning correctly, contact QIAGEN Technical Services.

Centrifoge

Make sure that the rotor and buckets are installed correctly.

All buckets must be mounted before starting a protocol run,

even if processing fewer than 12 samples. If the rotor or

buckets show signs of mechanical damage or corrosion, do

not use the QIAcube; contact QIAGEN Technical Services.

CAUTION

Damage to the instrument

[C4]

The QIAcube must not be used if the centrifuge lid is broken or if the lid lock is damaged.

Make sure that the rotor is installed correctly and that all buckets are properly mounted, even if processing fewer than 12 samples. Load the rotor symmetrically.

Only use rotors, buckets, and consumables designed for use with the QIAcube. Damage caused by use of other consumables will void your warranty.

In case of breakdown, caused by power failure, the centrifuge lid can be manually opened to remove the samples (see Section 7.2.7, page 7-15).

WARNING

Moving parts

[W12]



In case of breakdown caused by power failure, remove the power cord and wait 10 minutes before attempting to manually open the centrifuge lid.

WARNING



Risk of personal injury and material dantage [W13]
Raise the centrifuge lid carefully. The lid is beavy and may cause injury if it falls down.

WARNING

Risk of overheating

W141



To ensure proper ventilation, maintain a minimum clearance of 10 cm at the sides and rear of the QIAcube. Slits and openings that ensure the ventilation of the QIAcube must not be covered.

1.8 Heat hazard

The QIAcube worktable contains a heated shaker.

WARNING

Hot surface

[W15]



The shaker can reach temperatures of up to 70°C (158°F). Avoid touching it when it is hot.

1.9 Translations of warnings and cautions

This subsection contains translations of the warnings and cautions used in this user manual. Each warning or caution has a reference number in square brackets at the top right of its box.

WARNING



The term WARNING is used to inform you about situations that could result in **personal injury** to you or other persons.

Details about these circumstances are given in a box like this one.

DE	WARNING (WARNUNG)
	WARNUNG weist auf Situationen und Umstände hin, die zu einer Verletzung des Benutzers oder anderer Personer führen können.
	Nähere Angaben zu der Art der Gefährdung und der Vermeidung solcher Situationen werden in einem Textfeld wie diesem neben der Warnung gemacht.
FR	WARNING (DANGER) La formule WARNING (DANGER) est utilisée pour avertir des situations pouvant occasionner des dornmages corporeis à l'utilisateur ou à d'autres personnes.
	Les détails sur ces circonstances sont données dans un encadré semblable à celui-ci.

WARNING	Risk of personal injury and tradferial damage [w1] Improper use of the QlAsube may cause personal injuries or damage to the instrument. The QlAcube must only be operated by qualified personnel who have been appropriately trained. Servicing of the QlAcube must only be performed by QlAGEN Field Service Specialists.
DE	Verleizungsgefahr und Beschädigung des Gerätes Die unsachgemäße Bedienung des QIAcube kann zu einer Verletzung des Benutzers oder zur Beschädigung des Gerätes führen. Die Bedienung des QIAcube darf nur durch qualifiziertes Personal das entsprechend geschult wurde, erfolgen. Die Wartung des QIAcube darf nur durch Mitarbeiter des QIAGEN Kundendienstes durchgeführt werden.
FR	Risque de dommages corporeis et matériels L'utilisation non convenable du QIAcube peut causer des blessures ou des détériorations de l'instrument. Le QIAcube ne doit être utilisé que par du personnel qualifié qui a été formé de façon appropriée. Seul un ingénieur du service après-vente QIAGEN est autorisé à effectuer des travaux d'entretien sur le QIAcube.

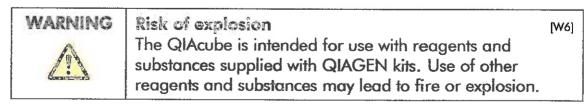
WARNING	Risk of personal injury and material damage [W2] The QIAcube is too heavy to be lifted by one person. To avoid personal injury or damage to the instrument, do not lift the instrument alone.
DE	Verletzungsgefahr und Beschädigung des Gerätes Der QIAcube ist zu schwer um von einer Person gehoben zu werden. Um Verletzungen des Benutzers oder eine Beschädigung des Gerätes zu vermeiden ist davon abzusehen, das Gerät alleine zu heben.
FR	Risque de dommages corporels et matériels Le QIAcube est trop lourd pour être soulevé par une personne. Pour éviter des dommages corporels ou matériels, ne pas soulever l'instrument tout seul.

WARNING	Risk of personal injury and material damage [W3] Do not attempt to move the QlAcube during operation.
DE	Verletzungsgefahr und Beschödigung des Gerätes Den QlAcube während eines Laufes nicht bewegen.
FR	Risque de dommages corporels et matériels Ne pas essayer de bouger le QIAcube pendant son fonctionnement.

WARNING	Electrical hazard	[W4]
A	Any interruption of the protective conductor (earth/grou	
14	lead) inside or outside the instrument or disconnection	of
Samuel A	the protective conductor terminal is likely to make the	
	Instrument dangerous.	
	Intentional interruption is prohibited.	
(Lethal voltages inside the instrument	
	When the instrument is connected to line power, termin	nals
	may be live, and opening covers or removing parts is I	
	to expose live parts.	

DE	Gefährdung durch Elektrizität Das Gerät muss zum Betrieb immer geerdet sein. Es ist verboten, die Schutzleiter im Gerät oder in der Netzzuleitung zu trennen oder zu entfernen. Gefährliche Spannung im Gerät Auch in ausgeschaltetem Zustand kann an einigen Stellen im Gerät Netzspannung anliegen, wenn das Gerät am Stromnetz angeschlossen ist. Das Öffnen oder Entfernen von Gehäuseteilen kann diese stromführenden Teile freilegen.
FR	Toute interruption du conducteur de protection à l'intérieur ou à l'extérieur de l'instrument, ou déconnexion du raccord du conducteur de protection (terre) peut rendre l'instrument dangereux. Il est interdit d'interrompre volontairement ce conducteur. Présence de tensions moctelles dans l'instrument Lorsque l'instrument est relié au secteur, les raccords peuvent être sous tension, et des parties sous tension peuvent être découventes en ouvrant des capots ou en retirant des pièces (à l'exception de celles auxquelles il est possible d'accéder manuellement).

WARNING	Explosive atmosphere [w5] The QIAcube is not designed for use in an explosive
DE	atmosphere. Explosionsfähige Atmosphären
	Der QIAcube darf nicht in explosionsfähigen Atmosphären betrieben werden.
PR S	Aimosphère explosive Le QlAcube n'est pas conçu pour fonctionner dans une atmosphère explosive.



DE	Explosionsgefahr Der QlAcube ist ausschließlich mit Reagenzien und Substanzen aus den QlAGEN Kits zu benutzen. Die Benutzung von anderen Reagenzien oder Substanzen kann Feuer oder eine Explosion auslösen.
FR	Risque d'explosion Le QlAcube a été conçu pour l'utilisation des réactifs et substances fournis par les kits QIAGEN. L'utilisation de réactifs et de substances autres que celles indiquées peut entrainer un risque d'incendie ou d'explosion.

WARNING



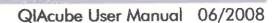
Samples containing infectious agents

[W7] Some samples used with this instrument may contain infectious agents. Handle such samples with the greatest of care and in accordance with the required safety regulations.

Always wear safety glasses, 2 pairs of gloves, and a lab

The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe, and that the instrument operators are suitably trained and not exposed to hazardous levels of infectious agents as defined in the applicable Material Safety Data Sheets (MSDSs) or OSHA, ACGIH, or COSHH documents.

Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.



DE	Infektiöses Probenmaterial Proben, die mit Hilfe dieses Gerätes prozessiert werden, können infektiöse Agenzien enthalten. Die Probenhandhabung sollte aus diesem Grund mit größter Vorsicht und gemäß den anzuwendenden Sicherheitsbestimmungen erfolgen. Es sollten immer Sicherheitsbrille, zwei Paar Handschuhe und ein Laborkittel getragen werden. Der Betreiber der Anlage ist für die Gewährleistung der Sicherheit am Arbeitsplatz verantwortlich. Er hat sicherzustellen, dass die Bediener des Gerätes ausreichend geschult sind und der Umgang mit infektiösen Agenzien nicht das in den Sicherheitsdaten brättern oder in anderen zu beachtenden Dokumenten testgelegte Ausmaß überschreitet. Bei der Behandlung von Ablutt und bei der Abfallbeseitigung sind alle gesetzlichen Regelungen zur Gesundheit und Sicherheit auf nationaler, regionaler und lokaler Ebene zu berücksichtigen.
FR	Echantillons contenant des agents infectieux Certains échantillons utilisés avec cet instrument peuvent contenir des agents infectieux. Manipuler ce type d'échantillon avec le plus grand soin et en accord avec les règles de sécurité requises. Toujours porter des lunettes de protection, deux paires de gants et une blouse de laboratoire. La personne responsable (par exemple le Chef du laboratoire) doit prendre les précautions nécessaires pour assurer la sécurité de l'environnement du poste de travail et pour être sûr que les opérateurs de l'instrument sont suffisamment formés et non exposés à des quantités dangereuses d'agents infectieux comme défini dans "Material Safety Data Sheets (MSDS)" ou des documents "OSHA, ACGIH ou COSHH". L'évacuation des vapeurs et déchets doit être conforme à tous règlements et dispositions légales - au plan national, départemental et local - concernant la santé et la sécurité.

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WARNING

i-lazardous chemicals

[W8]



Some chemicals used with this instrument may be hazardous or may become hazardous after completion of the protocol run.

Always wear safety glasses, gloves, and a lab coat. The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Material Safety Data Sheets (MSDSs) or OSHA, ACGIH, or COSHH documents.

Venting for fumes and disposal of wastes must be in accordance with all national, state, and local health and safety regulations and laws.

DE Gefährliche Chemikalien

Einige der in Verbindung mit diesem Gerät verwendeten Chemikalien sind gesundheitsgefährdend oder können nach Beendigung eines Protokoll-Durchlaufes gesundheitsgefährdend werden.

Es sollten immer Sicherheitsbrille, Handschuhe und ein Laborkittel getragen werden.

Der Betreiber der Antage ist für die Gewährleistung der Sicherheit am Arbeitsplatz verantwortlich. Er hat sicherzustellen, dass die Bediener des Gerätes ausreichend geschuft sind und nicht gesundheitsgefährdenden Konzentrationen toxischer Substanzen (chemischer oder biologischer) ausgesetzt sind, so wie dies in den Sicherheitsdatenblättern oder in anderen zu beachtenden Dokumenten festgelegt ist.

Bei der Behandlung von Abluft und bei der Abfallbeseitigung sind alle gesetzlichen Regelungen zur Gesundheit und Sicherheit auf nationaler, regionaler und lokaler Ebene zu berücksichtigen.

	T
FR	Substances chimiques dangereuses Certaines substances chimiques utilisées avec cet
	instrument peuvent être dangereuses ou peuvent le devenir après que le protocole ait été effectué.
	Toujours porter des lunettes de protection, paire de gants et une blouse de laboratoire.
	La personne responsable (par exemple le Chef du
	laboratoire) doit prendre les précautions nécessaires pour
	assurer la sécurité de l'environnement du poste de travail
	et pour être sûr que les opérateurs de l'instrument sont
	suffisamment formés et non exposés à des quantités
	dangereuses de substances toriques (chimique ou
	biologique) comme défini dans "Material Safety Data
	Sheets (MSDS)" ou des documents "OSHA, ACGIH ou
	COSHH".
	L'évacuation des vapeurs et déchets doit être conforme à
	tous règlements et dispositions légales - au plan national,
	départemental et local - concernant la santé et la sécurité.

WARNING	When cleaning the QlAcube with alcohol-based disinfectant, leave the QlAcube door open to allow flammable vapors to disperse. Only clean the QlAcube when worktable components have cooled down.
DE	Peuergefahr Beim Reinigen des QIAcube mit einem auf Alkohol basierenden Desinfektionsmittel muss die Tür des QIAcubes offen gelassen werden, damit die brennbaren Dämpfe entweichen können. Den QIAcube nur reinigen, sobald die entsprechenden Module auf der Arbeitsfläche abgekühlt sind.
FR	Risque de fau Lors du nettoyage du QIAcube avec un désinfectant à base d'alcool, laisser la porte du QIAcube ouverte pour permettre aux vapeurs inflammables de s'évaporer. Nettoyer le QIAcube uniquement quand les composants de la table de travail ont refroidi.

WARNING	Toxic fumes Do not use bleach to disinfect used labware. Bleach in contact with salts from the buffers used can produce toxic fumes.
DE	Giftige Dämpfe/Gase Für die Reinigung oder Desinfektion des Gerätes dürfen keine Bleichmittel verwendet werden. Durch den Kontakt von Bleichmitteln mit den Salzen der verwendeten Pufferlösungen kann es zur Bildung giftiger Gase ader Dämpfe kommen.
FR	Vapeurs toxiques Ne pas utiliser de l'eau de javel pour nettever ou désinfecter l'instrument. L'eau de javel en contact avec des tampons salins peut produire des fumées toxiques.

WARNING	Moving parts To avoid contact with moving parts during operation of the QIAcube, the instrument must be operated with the door closed. If the sensor is not functioning correctly, contact QIAGEN Technical Services.
DE	Bewegliche Geräteteile Während des Gerätelaufs ist jeglicher Kontakt mit den beweglichen Geräteteilen zu vermeiden; das Gerät darf ausschließlich bei geschlossener Tür in Betrieb genommen werden. Sollte der Sensor nicht ordnungsgemäß funktionieren, kontaktieren Sie bitten den Technischen Service von QIAGEN.
FR	Lorsque le QIAcube est en marche, toujours fermer la porte de l'instrument afin d'éviter tout contact avec les éléments mobiles de l'appareil. Si le détecteur ne fonctionne pas correctement, contacter le Support Technique QIAGEN.

WARNING	Moving parts In case of breakdown caused by power failure, remove the power cord and wait 10 minutes before attempting to manually open the centrifuge lid.
DE	Bewegliche Geräteteile Bei einem durch Stromausfall entstandenen Ausfall des Gerätes das Stromkabel entfernen und 10 Minuten warten bevor der Zentrifugendeckel manuell geöffnet werden kann.
FR	Eléments mobiles Dans le cas d'un arrêt causé par une panne de courant, retirer le câble d'alimentation et attendre 10 minutes avant d'essayer d'ouvrir manuellement le couvercle de la centrifugeuse.

WARNING	Risk of personal injury and material damage [W13] Raise the centrifuge lid carefully. The lid is heavy and may cause injury if it falls down.
DE	Verletzungsgefahr und Beschädigung des Gerätes Den Zentrifugendeckel vorsichtig anheben. Der Deckel ist schwer und kann Verletzungen verursachen falls er herunterfällt
FR	Risque de dommages corporels et matériels Soulever le couvercle de la centrifugeuse avec précaution. Le couvercle est lourd et peut causer des blessures si il tombe.

WARNING
Risk of overheating
To ensure proper ventilation, maintain a minimum
Plearance of 10 cm at the sides and rear of the QIAcube.
Slits and openings that ensure the ventilation of the QIAcube must not be covered.

DE	Überhitzung des Gerätes Zur Sicherstellung einer ausreichenden Belüftung des QIAcube muss ein Mindestabstand von 10 cm an den Seiten und an der Rückseite des Gerätes eingehalten werden. Lüftungsschlitze und -öffnungen des Gerätes nicht abdecken.
FR	Risque de surchauffe Laisser un espace d'au moins 10 cm sur les côtés et à l'arrière du QIAcube pour assurer une ventilation efficace. Les grilles et prises d'air assurant la ventilation du QIAcube ne doivent pas être couvertes.

WARNING	Hot surface The shaker can reach temperatures of up to 70°C (158°F). Avoid touching it when it is hot
DE	Heiße Oberfläche Der Schüttler kann Temperaturen bis zu 70°C (158°F) erreichen. Vermeiden Sie es den Schüttler zu berühren, solange er heiß ist.
FR	Surface brûlante L'agitateur peut atteindre des températures jusqu'à 70°C (158°F). Eviter de le toucher quand il est chaud.

WARNING	Risk of personal injury and material damage [W16]
A	Do not use damaged rotor adapters. The rotor adapters
	are for single use only. Do not reuse the rotor adapters as
4 december 2	they can be damaged by the high g forces experienced in
/	the centrifuge.
DE	Verleizungsgefahr und Beschädigung des Gerätes
	Benutzen Sie keine beschädigten Rotoradapter.
	Rotoradapter sind nur für den Einmalgebrauch. Benutzen
	Sie die Rotoradapter kein zweites Mal, da sie durch die
	hohen g Kräfte bei der Zentrifugation beschädigt werden
	können.
	Romien.

FR	Risque de dommages corporels et matériels Ne pas utiliser d'adaptateur à rotor endommagé. Les adaptateurs à rotor sont faits que pour une seule utilisation. Ne pas les utiliser une deuxième fois car ils peuvent être endommagés par les hautes forces g de la centrifugation.
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WARNING	Risk of personal injury and material damage [w17] The rotor nut must be securely tightened using the rotor key supplied with QIAcube. If the rotor nut is not tightened properly, it can become loose during operation of the centrifuge.
DE	Verletzungsgefahr und Deschädigung des Gerätes Die Rotorschraube muss zur Sicherheit mit dem Rotorschlüssel angezogen werden, der zusammen mit dem QIAcube geliefert wird. Sollte die Schraube nicht fest genug angezogen sein, kann sie sich während der Zentrifugation lösen.
FR	Risque de dommages corporels et matériels La vis du rotar doit être proprement serrée à l'aide de la clé du rotar fournie avec le QIAcube. Si la vis n'est pas assez serrée, elle peut se desserrer lorsque la centrifugeuse est en marche.

WARNING	Misk of personal injury and material damage [W18] Make sure that lids from spin columns and 1.5 ml microceptrifuge tubes are in the correct position and pushed all the way down to the bottom of the slots on the sides of the of the rotor adapter. Incorrectly positioned lids can break off during centrifugation.
	Merleizungsgefahr und Beschädigung des Gerätes Stellen Sie sicher, dass die Deckel von den Spin-Säulen und den 1,5 ml Mikrozentrifugationsröhrchen in der korrekten Position angebracht sind und bis zum Boden des Deckelschachts an den Seiten des Rotor-Adapters durchgedrückt sind. Falsch positionierte Deckel können während der Zentrifugation abgerissen werden.

FR	Risque de dommages corporels et matériels
	Assurez-vous de positionner correctement les couvercles
	des colonnes de centrifugation et des tubes de 1,5 ml et de
	les enfoncer jusqu'au bout de l'emplacement sur les bords
	de l'adaptateur à rotor. Les couvercles placés
	incorrectement peuvent se casser lors de la centrifugation.

WARNING	Risk of personal injury and material damage [W19] Be sure the lid is completely removed from the spin column. Spin columns with partially removed lids may not be removed properly from the rotor, causing the protocol run to crash.
DĒ	Verletzungsgefahr und Beschädigung des Gerätes Stellen Sie sicher, dass der Deckel von der Spin-Säule abgetrennt ist. Spin-Säulen mit unvollständig entfernten Deckeln lassen sich nicht korrekt aus dem Rotor entnehmen, was einen Protokollabbruch zur Folge hätte.
FR	Risque de dommages corporels et matériels Assurez-vous d'enlever les couvercles des colonnes de centrifugation. Les colonnes de centrifugations avec un couvercle partiellement enlevé se détachent difficilement du rotor ce qui est la cause d'un crash du protocole.

Risk of material damage [W20]	
Do not use 1.5 ml microcentrifuge tubes on the shaker.	
Because 1.5 ml microcentrifuge tubes can cause filter-tips	
to stick during sample transfer, their use on the shaker can	
damage the pipetting system and can cause a centrifuge	
erash.	
Beschädigung des Gerätes	
Benutzen Sie keine Mikrozentrifugationsröhrchen (1,5 ml)	
auf dem Schüttler. Mikrozentrifugationsröhrchen können	
daze führen, dass Filter-Tips während des Probentransfers	
steckenbleiben. Die Benutzung dieser Röhrchen auf dem	
Schüttler kann daher das Pipettiersystem beschädigen und	
einen Zentrifugenunfall verursachen.	

FR	Risque de dommages matériels Ne pas utiliser de tubes de centrifugation d'1,5 ml sur l'agitateur. Pendant le transfert des échantillons les tubes de centrifugation peuvent bloquer les cônes à filtre. L'usage des tubes sur l'agitateur peut donc abîmer le système de pipetage et causer une panne de la centrifugeuse.
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WARNING	Risk of electric shock Do not open any panels on the QIAcube. Risk of personal injury and professal damage Only perform maintenance that is specifically described in this user manual.
DE	Gefährdung durch Elektriziföt Unter keinen Umständen darf das Gehäuse des QIAcube geöffnet werden. Verletzungsgefahr und Beschädigung des Gerätes Keine Pflege- und Wartungsarbeiten durchführen, die nicht in diesem Handbuch beschrieben sind.
FR	Risque d'électrocution Ne pas ouvrir les panneaux du QIAcube. Risque de dommages personnels et matériels Réaliser uniquement la maintenance décrite spécifiquement dans ce manuel.

Hazardous chemicals and infectious agents [w22] The waste contains samples and reagents. This waste may sontain toxic or infectious material and must be disposed of properly. Refer to your local safety regulations for proper disposal procedures. Gefährliche Chemikalien und infektiöse Agenzien Der Abfall kann gesundheitsgefährdende Reagenzien oder infektiöses Probenmaterial enthalten und muss gemäß den lokalen Sicherheitsvorschriften entsorgt werden.

FR	Agents infectieux et substances chimiques dangereuses
	Les déchets contiennent des échantillons et des réactifs. Ils peuvent contenir des agents infectieux et doivent être éliminés selon les règles de sécurité de votre laboratoire.

WARNING	Risk of personal injury [W23] After a plastic crash, sharp plastic particles could be inside the centrifuge. Be careful when handling items inside the centrifuge.
DE	Verletzungsgefahr Sollten Plastikteile beschädigt sein, können sich scharfe Plastikstücke innerhalb der Zentrifuge befinden. Bei der Benutzung von Gegenständen innerhalb der Zentrifuge ist daher Vorsicht angebracht.
FR	Risque de dommages corporels Si du plastique s'est cassé, des morceaux pointus peuvent se trouver à l'intérieur de la centrifugeuse. Soyez attentifs lors de la manipulation de matériel à l'intérieur de la centrifugeuse.

CAUTION	The term CAUTION is used to inform you about situations that could result in damage to the workstation or other equipment. Details about these circumstances are given in a box like this one.
DE	CAUTION (ACHTUNG) ACHTUNG weist auf Situationen und Umstände hin, die zu
	einer Beschädigung des Gerätes führen können. Um einen Geräteschade n zu vermeiden, muss die genannte Anteitung unbedingt befolgt werden.
	Nähere Angaben zu der Art der Gefährdung und der Vermeidung solcher Situationen werden in einem Textfeld wie diesem gemacht.

FR	CAUTION (ATTENTION) Le terme CAUTION (Attention) est utilisé pour signaler les situations susceptibles de provoquer des détériorations de l'instrument ou d'autre matériel. Les détails sur ces circonstances figurent dans un encadré semblable à celui-ci.
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CAUTION	Damage to the instrument Avoid spilling water or chemicals crite the QIAcube. Damage caused by water or chemical spillage will void your warranty.
DE	Beschädigung des Gerätes Vermeiden Sie es, Wasser oder Chemikalien auf dem QlAcube zu verschütten. Durch verschüttetes Wasser oder verschüttete Chemikalien verusachte Geräteschäden sind nicht durch die Garantie abgedeckt.
FR	Déterioration de l'appareil Eviter de renverser de l'eau ou des substances chimiques sur le QIAcube. Tout donnage causé par de l'eau ou des produits chimiques mettra fin à la garantie.

CAUTION	Damage to the instrument [C2] Only use QIAGEN spin columns and QIAGEN Spin Kits with the QIAcube. Damage caused by use of other types of spin columns or chemistries will void your warranty.
DE	Beschädigung des Gerätes Es dürfen ausschließlich QIAGEN Spin-Säulen und QIAGEN Spin-Kits auf dem QIAcube benutzt werden. Geräteschäden, die durch die Verwendung von anderen Spin-Säulen oder Chemie-Typen verursacht werden, sind Aicht durch die Garantie abgedeckt.
FR	Déterioration de l'instrument Utiliser uniquement des colonnes de centrifugation et des kits QIAGEN avec le QIAcube. Tout dommage causé par l'utilisation d'un autre type de colonnes ou de chimie mettra fin à la garantie.

CAUTION	Damage to the instrument Direct sunlight may bleach parts of the instrument and cause damage to plastic parts. The QIAcube must be located out of direct sunlight.
DE	Beschädigung des Gerätes Direktes Sonnenlicht kann Teile des Gerätes bleichen und Plastikteile schädigen. Der QIAcube darf nicht ins direkte Sonnenlicht gestellt werden.
FR	Détérioration de l'instrument La lumière directe du soleil peut décolorer des parties de l'instrument et endommager des parties en plastique. Placer le QIAcube en dehors de la lumière directe du soleil.

CAUTION	Damage to the instrument The QIAcube must not be used if the centrifuge lid is broken or if the lid lock is damaged. Make sure that the rotor is installed correctly and that all buckets are properly mounted, even if processing fewer than 12 samples. Load the rotor symmetrically. Only use rotors, buckets, and consumables designed for use with the QIAcube
DE	Beschädigung des Cerätes Bei Beschädigung des Zentrifugendeckels oder Zentrifugenschlosses darf der QlAcube nicht benutzt werden. Stellen Sie sicher, dass der Rotor korrekt installiert ist und dass sämtliche Zentrifugenbecher ordnungsgemäß montiert sind, auch bei Verarbeitung von weniger als 12 Proben. Den Rotor symmetrisch beladen. Ausschließlich Rotoren, Zentrifugenbecher und Reagenzien benutzen, die auf den QlAcube angepasst sind.

FR	Dommage de l'appareil Ne pas utiliser le QIAcube lorsque le couvercle de la centrifugeuse est cassé ou que la fermeture du couvercle est endommagée. S'assurer que le rotor est installé correctement et que tous les godets sont bien montés, même si vous traitez moins de 12 échantillons. Charger le rotor symétriquement.
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CAUTION	Damage to the instrument Do not use bleach, solvents, or reagents containing acids, alkalis, or abrasives to clean the QLAcube.
DE	Beschädigung des Gerätes Es dürfen weder Bleiche noch säure- oder laugehaltige Reinigungs- oder Scheuermittel behutzt werden.
FR	Déterioration de l'appareil Ne pas utiliser d'eau de Jave), de solvants ou de réactifs contenant des asides, des substances alcalines ou abrasives pour nettoye le QIAcube.

CAUTION	Damage to the instrument Do not use detergents, alcohol, or alcohol-based disintectarits to clean the QIAcube door.	C6]
DE	Beschädigung des Gerätes Zur Reinigung der QIAcube Tür keine Detergenzien oder auf Alkohol basierenden Reinigungsmittel benutzen.	
FR	Détérioration de l'appareil Ne pas utiliser de détergents ou de désinfectants à base d'alcool pour nettoyer la porte du QIAcube.	

1.10 Symbols on the QIAcube

Symbol	Location	Language	Description
	Shaker	EN	Heat hazard — the temperature of the shaker can reach up to 70°C (158°F).
	Schüttler	DE	Verbrennungsgefahr — die Temperatur des Schüttlers kann bis zu/70°C (158°C) heiß werden
	Agitateur	FR (Risque de brûlures — la température de l'agitateur peut atteindre jusqu'à 70°C (158°F).
A	Near the centrifuge; near the robotic arm	EN	Mechanical hazard — avoid contact with moving parts.
	Nahe der Zentrifuge; nahe des Roboterarms	DE S	Mechanische Gefahr — Kontakt mit beweglichen Geräteteilen vermeiden.
	Près de la centrifugeuse; près du bras robotique	FR	Danger mécanique — éviter d'entrer en contact avec les parties mobiles.

Symbol	Location	Language	Description
	Inside the waste drawer	EN	Biohazard — the waste drawer may be contaminated with biohazardous material and must be handled with gloves.
	Innerhalb des Abfallbehälters	DE \$\sqrt{7}\$	Biologische Gefährdung — der Abfallbehälter kann durch biologisch gefährliches Material kontaminiert sein und muss mit Handschuhen angefasst werden.
	A l'intérieur du compartiment à déchets	F //	Danger Biologique — le compartiment à déchets peut être contaminé par du matériel présentant un risque biologique et doit être manipulé avec des gants.
CE	Type plate on the back of the instrument	EN 7	CE mark
	Plakette auf der Röckseite des Geräfes	DE	CE-Zeichen
	Plaque à l'arrière de l'appareil	FR	Marquage CE

Symbol	Lecation	Language	Description
c Us	Type plate on the back of the instrument	EΝ	CSA listing mark for Canada and the USA
	Plakette auf der Rückseite des Gerätes	DE	CSA-Zeichen für Kanada und die USA
	Plaque à l'arrière de l'appareil	FR	Marquage GSA pour le Canada et les Etats-Unis
СВ	Type plate on the back of the instrument	EN	CB mark of the IECEE member states
	Plakette auf der Rückseite des Gerätes	DE	CB-Zeichen der IECEE- Mitgliedsstaaten
	Plaque à l'arrière de l'appareil		Marquage CB des Etats membres de l'IECEE
FC	Type plate on the back of the instrument	EN	FCC mark of the United States Federal Communications Commission
	Plakette auf der Rückseite des Gerätes	DE	FCC Markierung der Federal Communications Kommission der USA
<	Plaque à l'arrière de l'appareil	FR	Marquage FCC de la commission fédérale des communications des Etats Unis

Symbol	Location	Language	Description
C	Type plate on the back of the instrument	EN	C-Tick mark for Australia (supplier identification N17965)
	Plakette auf der Rückseite des Gerätes	DE	C-Tick Markierung für Australien (Hersteller- Identifikation N17965)
	Plaque à l'arrière de l'appareil	FR	Etiquette C-Tick pour l'Australie (Identification du fournisseur N17965)
25	Type plate on the back of the instrument	EN	RollS mark for China (the restriction of the use of gertain hazardous substances in electrical and electronic equipment)
	Plakette auf der Rückseite des Gerätes	7	RoHS Markierung für China (Beschränkung der Verwendung bestimmter gefährlicher Stoffe in Elektro- und Elektronikgeräten)
	Plaque à l'arrière de l'appareil	FR	Marquage RoHS pour la Chine (limitation de l'utilisation de certaines substances dangereuses dans les équipements électriques et électroniques)
			. , , , , , , , , , , , , , , , , , , ,

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Symbol	Location	Language	Description
X	Type plate on the back of the instrument	EΝ	WEEE mark for Europe
	Plakette auf der Rückseite des Gerätes	DE	WEEE-Zeichen für Europa
	Plaque à l'arrière de l'appareil	FR	Marquage WEEE pour l'Europe

2 Introduction

Thank you for choosing the QIAcube. We are confident it will become an integral part of your laboratory.

Before using the QIAcube, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

2.1 About this user manual

This user manual provides information about the QIAcube in the following sections:

- 1. Safety Information
- 2. Introduction /
- 3. General Description
- 4. Installation Procedures
- 5. Operating Procedures
- 6. Maintenance Procedures
- 7. Troubleshooting
- 8. Glossary

Appendices

Index

The appendices contain the following:



hatormation about loading the centrifuge and shaker

QIAcube accessories

Liability clause



2.2 General information

2.2.1 Technical assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QIAcube or QIAGEN products in general, do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore engaging you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, call one of the QIAGEN Technical Services Departments or local distributors (see back cover).

For up-to-date information about the QlAcube, including new protocols for download, visit www.qiagen.com/MyQlAcube

2.2.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual. Please contact QIAGEN Technical Services.

2.2.3 Version management

This document is the QIAcube User Manual, version 1.1.

Intended use of the QIAcube 2.3

The QIAcube is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users, such as technicians and physicians trained in molecular biological techniques and the operation of the QIAcube.

The QlAcube is intended to be used only in combination with QIAGEN kits indicated for use with the CHAcube for the applications described in the kit bandbooks.

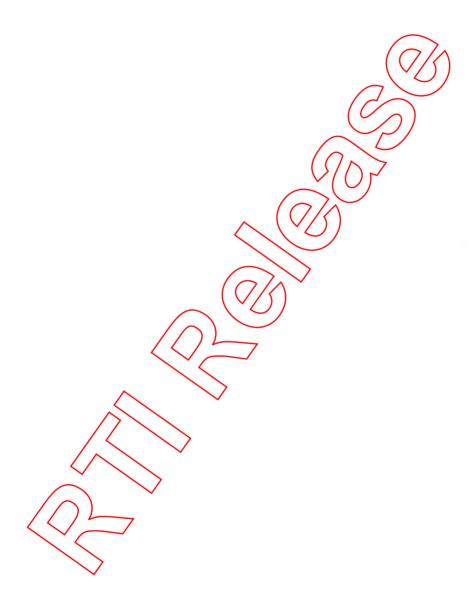
2.3.1 Requirements for QIAcube users

The table on the next page coyers the general level of competence and training necessary for transportation, installation, use, maintenance, and servicing of the QIAcube.

Task	Personnel	Training and experience
Transportation	No special requirements	No special requirements
Installation	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Routine use (running protocols)	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Maintenance procedures	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Servicing	QIAGEN Field Service Specialists only	

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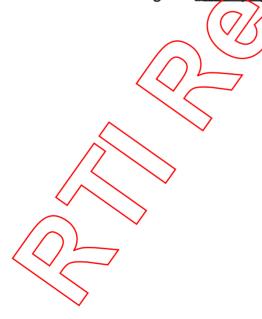


3 General Description

The QlAcube performs fully automated processing of up to 12 samples. The instrument controls integrated components including a centrifuge, heated shaker, pipetting system, and robotic gripper.

The QIAcube is preinstalled with various protocols for processing QIAGEN spin columns for purification of RNA, genomic DNA, plasmid DNA, viral nucleic acids, or proteins, plus DNA and RNA cleanup. The user selects a protocol using the touchscreen, and loads plasticware, samples, and reagents onto the QIAcube worktable. The user then closes the instrument door and starts the protocol, which provides all necessary commands for sample tysis and purification using QIAGEN spin columns. A fully automated load check helps to ensure correct loading of the worktable.

The range of protocols available is continually expanding, and additional QIACEN protocols can be downloaded free of charge at www.giagen.com/MyQIAcube

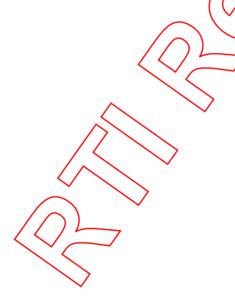


3.1 QIAcube principle

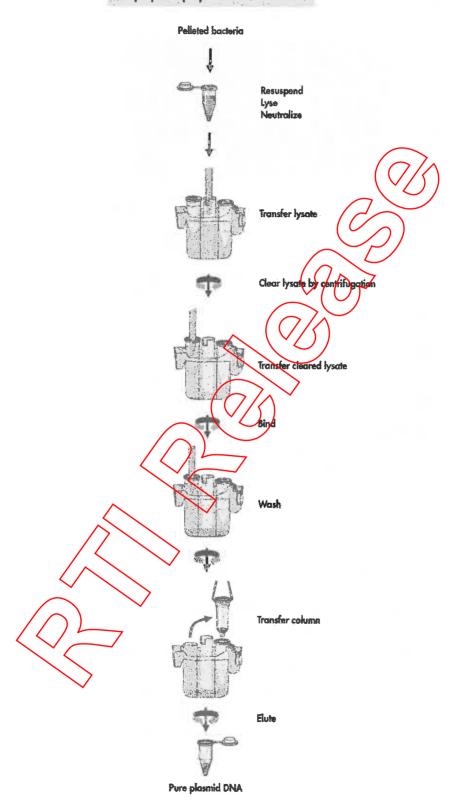
Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute). No change in purification chemistry is required as you simply continue to use trusted QIAGEN spin-column kits.

- Samples are lysed in the orbital shaker, which can be heated if required by the protocol.
- 2. Each lysate is transferred to a spin column in a retor adapter. If the lysate needs to be homogenized or cleared, it is first transferred to the middle position of the rotor adapter.
- 3. Nucleic acids or proteins bind to the silica membrane or purification resin of the QIAGEN spin column and are washed to remove contaminants.
- 4. The spin column is transferred to a microcentrifuge tube for elution of purified nucleic acids or protein.

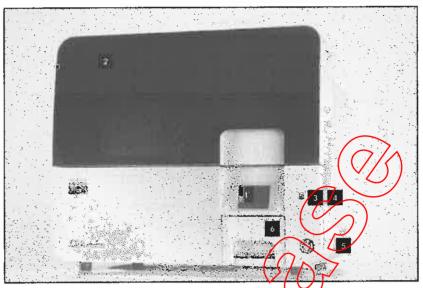
For an example workflow using a QIAGEN spin-column kit, see the flowchart on the next page.



GIAprep Miniprep Standard Procedure



3.2 External features of the QIAcube



Front view of the QIAcube.

- Touchscreen
- USB port behind protective panel

Door

- Power switch
- RS232 serial port behind protective panel (for use by QIAGEN Field Service Specialists only)
- Waste drawer

Touchscreen

The touchscreen allows the user to select and run protocols, install or delete protocols, save data files, and operate the shaker and centrifuge individually. Messages displayed in the touchscreen guide the user through worktable setup after a protocol has been selected. During sample processing, the touchscreen shows the protocol status and remaining time. For convenience and ease of use, the names of the last 2 protocols performed are displayed in the main menu, enabling rapid selection of frequently used protocols.

Button	Description	
Cleanup	Displays protocols for clean up of DNA from amplification reactions and agarose gels or clean up of RNA	
DNA	Displays protocols for genomic or plasmid DNA purification	
Protein	Displays protocols for protein purification and protein depletion	
RNA	Displays protocols for RNA purification	
Virus	Displays protocols for viral nucleic acid purification	
Tools	Displays additional functions	

Door

The QIAcube door protects users from the moving robotic arm and from potentially infectious material placed on the worktable. The door can be manually opened to gain access to the worktable. During operation of the QIAcube, the door must remain closed and should only be opened when instructed to do so by the software. If the door is opened during a protocol run, the protocol run will immediately stop and an error message will be displayed in the touchscreen. The samples must then be processed manually.

RS232 serial port

The RS232 serial port, located behind the protective panel, is for use by QIAGEN Field Service Specialists only.

USB port

The USB port, located behind the protective panel, allows connection of the QIAcube to a USB stick (supplied with the QIAcube). New protocols and software versions, available at www.qiagen.com/MyQIAcube, can be downloaded to the USB stick and transferred to the QIAcube via the USB port. Data files, such as log files or report files can also be

transferred via the USB port from the QIAcube to the USB stick.

Important: The USB port is only for use with the USB stick provided by QIAGEN. Do not connect other devices to this port.

Important: Do not remove the USB stick while downloading protocols or software or transferring data files or during a protocol run.

Power switch

The power switch is located at the front (ight of the QIAcube. To switch the QIAcube on, press the switch. The beeper sounds, and the startup screen appears. The instrument then automatically performs initialization tests.)

To conserve energy, the QIAcube can be switched off when not in use. To switch off the QIAcube, press the switch.

Waste drawer

Used disposable filter-tips are discarded through 2 slots in the worktable and collected in the waste drawer. Used columns (e.g., Q/Ashredder columns) are also discarded into this drawer.

Power cord socket

The power cord socket is located at the rear right of the QIAcube and allows connection of the QIAcube to a power outlet via the supplied power cord.

Cooling air outlet

Cooling air outlets are located at the left side of the QIAcube and allow cooling of the internal components of the QIAcube.

3.3 Internal features of the QIAcube



Internal view of the Clacabe.

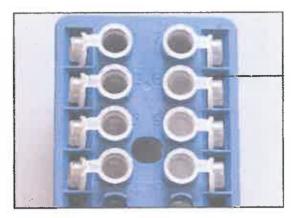
- Centrifuge (lid/
- Centrifuge
- Shaker
- Reagent bottle rack
- Tip sensor

- Microcentrifuge tube slots
- Tip racks
- Disposal slots for tips and columns
- Robotic arm

Shoker

The heated orbital shaker enables fully automated lysis of up to 12 samples. Shaker adapters are available for 2 ml microcentrifuge tubes and 2 ml screw-cap tubes. Sample tubes are placed into a rack that fits onto the shaker adapter. The lid of each microcentrifuge tube or shaker rack plug of each screw-cap tube is held in a slot at the edge of the shaker rack. This ensures that microcentrifuge tubes cannot be displaced during sample processing and allows shaker

loading to be checked. The positions on the shaker rack are numbered to enable easy loading.



Sample tube lids are held in slots at the edge of the shaker rack

Shaker rack.

The shaker can also be operated individually via the touchscreen.

Centrifuge

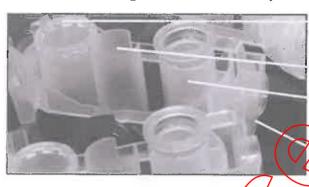
The centrifuge is equipped with 12 swing-out buckets, each of which can hold a disposable rotor adapter. Up to 12 samples can be processed per run. For ease of use and high process safety, a gray line marks the side of the bucket that must face toward the center of the rotor. All centrifuge buckets must be mounted before starting a protocol run, even if fewer than 12 samples are to be processed. For more information, see Appendix B or the loading chart available at www.giagen.com/MyQlAcube.

The centrifuge can also be operated individually via the touchscreen.

Rojor adapter

A disposable rotor adapter holds a QIAGEN spin column and a microcentrifuge tube in a centrifuge bucket during sample processing. If required by the protocol, an additional column (e.g., QIAshredder column) can be placed in the middle position of the rotor adapter. For ease of use and high process safety, the rotor adapters are designed so that they fit into a centrifuge bucket only in the correct orientation.

Spin column and microcentrifuge tube lids are held securely in slots at the edge of the rotor adapter.



Microcentrifuge tube position

Middle position

Wash position

Slot for spin-column

Novel rotor adapters.

The wash position of the rotor adapter is open at the bottom, enabling wash buffers to flow through and collect at the bottom of the rotor adapter during centrifugation. The other 2 positions in the rotor adapter are closed. If processing fewer than 12 samples, make sure to load the rotor symmetrically with rotor adapters. For more information, see Appendix B or the loading chart available at www.qiagen.com/MyQlAcube.

WARNING



Risk of personal injury and material damage [W16]
Do not use damaged rotor adapters. The rotor adapters are for single use only. Do not reuse the rotor adapters as they can be damaged by the high g forces experienced in the centrifuge.

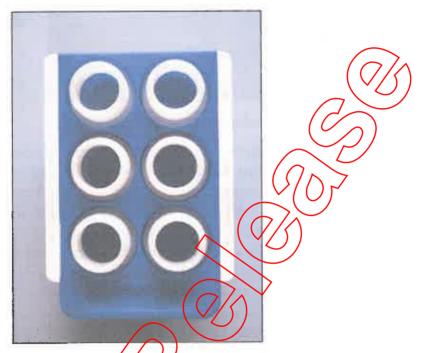
keagent bottle rack



The reagent bottle rack holds up to six 30 ml reagent bottles and, for ease of use and high process safety, fits onto the Alacube worktable only in the correct orientation. Liquid is aspirated from the bottles by the pipetting system. An application-specific labeling strip must be attached to the reagent bottle rack. For increased convenience and ease of use, the labeling strip fits only in the correct orientation on the reagent bottle rack. The use of the labeling strip allows easy identification of buffer positions and ensures that the

rack is correctly positioned on the worktable for liquid-level detection.

Note: Reagent bottles designed for use with the QIAcube must be used otherwise errors may occur during liquid detection.



Reagent bottle rack with labeling strips.

Tip racks

Two tip racks can be placed on the QIAcube worktable. Tips can be purchased in prefilled tip racks holding 200 μ l filter-tips, 1000 μ l filter-tips, or 1000 μ l wide-bore filter-tips. Two tip racks can be placed on the QIAcube.

Note: Filter-tips designed for use with the QIAcube must be

Microcentrifuge tube slots

In addition to the 12 tubes that the shaker can accommodate, up to 3 additional microcentrifuge tubes can be used in the microcentrifuge accessory position. These slots can be used if, for example, proteinase K or other enzyme is required for the purification protocol.

Tip disposal slots

Disposable filter-tips are alternately discarded through each of the tip disposal slots into the waste drawer. This prevents discarded tips from piling up in the waste drawer.

Column disposal slot

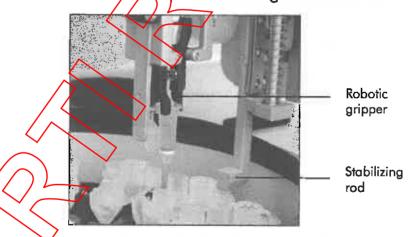
Used columns (e.g., QlAshredder columns) are discarded through this slot into the waste drawer.

Robotic arm

The robotic arm provides accurate and precise positioning of the robotic gripper and pipetting system on the QIAcube worktable and also includes the optical and ultrasonic sensor.

Robotic gripper

The robotic gripper transfers spin columns during sample preparation. During transfer of a spin column, a stabilizing rod holds the rotor adapter in place, ensuring it remains properly seated in the centrifuge bucket. The robotic gripper is behind the panel covering the robotic arm.



Fully automated spin-column processing.

Pipetting system

The QlAcube is equipped with a single-channel pipetting system that moves in the X, Y, and Z directions. The dilutor,

fitted with a tip adapter, is connected to a precision syringe pump, which enables accurate transfer of liquids. The tip adapter allows aspiration and dispensing of liquid through an attached disposable tip. Disposable filter-tips (200 μ l, 1000 μ l, or 1000 μ l wide-bore) are used for sample preparation to minimize the risk of cross-contamination.

Optical sensor

During the load check, the optical sensor checks that the number of rotor adapters corresponds to the humber of samples in the shaker and that both shaker and rotor are correctly loaded. The optical sensor also checks the type of tips loaded on the worktable and whether there are sufficient tips for the protocol run.

Note: The shaker and centrifuge must be symmetrically loaded (see Appendix B or the loading chart available at www.aiagen.com/MyQlAcube).

Ultrasonic sensor

During the load check, the plingsonic sensor checks that the buffer bottles in the reagent bottle rack contain sufficient buffer for the protogol run.

Tip sensor

During sample preparation, the tip sensor checks that the tip adapter has picked up a tip and checks whether it is a $200 \mu l$, or a $1000 \mu l$ type filter-tip.



4 Installation Procedures

This section provides instructions on unpacking, packing, and installing the QIAcube.

4.1 Requirements

Site

The QIAcube must be located out of direct sunlight, away from heat sources, and away from sources of vibration and electrical interference. Refer to Appendix A for the operating conditions (temperature and humidity) The site of installation should be free of excessive drafts, excessive moisture, and excessive dust and should not be subject to large temperature fluctuations.

Use a level workbench that is large enough and strong enough to accommodate the QIAcube. Refer to Appendix A for the weight and dimensions of the QIAcube.

Ensure that the workbench is dry, clean, and vibration-proof and has additional space for accessories.

The Qlacube must be placed within approximately 1.5 m of a properly grounded (earthed) AC power outlet. The power line to the instrument should be voltage regulated and surge protected.

WARNING

Explosive atmosphere

[W5]

The QIAcube is not designed for use in an explosive atmosphere.

WARNING

Kisk of overheating

[W14]

To ensure proper ventilation, maintain a minimum clearance of 10 cm at the sides and rear of the QiAcube. Slits and openings that ensure the ventilation of the QIAcube must not be covered.

4.2 AC power connection

Power requirements

The QiAcube operates at:

- 100–120 V AC, 50/60 Hz, 650 VA (North America and Japan)
- 220–240 V AC, 50/60 Hz, 650 VA (Europe)

Make sure that the voltage rating of the QlAcube is compatible with the AC voltage available at the installation site. Mains supply voltage fluctuations are not to exceed 10% of nominal supply voltages.

Grounding requirements

To protect operating personnel, the National Electrical Manufacturers' Association (NEWA) recommends that the QIAcube be correctly grounded (earthed). The instrument is equipped with a 3-conductor AC power cord that, when connected to an appropriate AC power outlet, grounds (earths) the instrument. To preserve this protection feature, do not operate the instrument from an AC power outlet that has no ground (earth) connection.

Installation of AC power cord

Connectione end of the AC power cord to the socket located at the rear of the QIAcube, and the other end to the AC power outlet.

4.3 Unpacking the QIAcube

WARNING



Risk of personal injury and material damage [W2] The QIAcube is too heavy to be lifted by one person. To avoid personal injury or damage to the instrument, do not lift the instrument alone.

Before unpacking the QIAcube, move the package to the site of installation and check that the arrows on the package point upward. In addition, check whether the package is damaged. In case of damage, contact the transporter of the package. The shockwatch/tiltwatch indicator should be white. If it is red, broken, or missing, contact the transporter of the package.

Open the top of the transportation box to remove the centrifuge rotor, QIAcube User Manual, and power cord before lifting the box. If the box is lifted before the rotor has been removed, the rotor could fall to the ground and become damaged.

Remove the black foam protector lid and lift up the box.

When lifting the QIAcube, slide your fingers under both sides of the workstation and keep your back straight.

After unpacking the QlAzube, check that the following documents are supplied:

- Packing list
- Warranty registration form
- QIAcube User Manual

Read the packing list to check that you have received all items. If anything is missing, contact QIAGEN Technical Services.

Check that the QIAcube is not damaged and that there are no loose parts. If anything is damaged, contact QIAGEN Technical Services. Make sure that the QIAcube has equilibrated to ambient temperature before operating it.

Retain the package in case you need to transport the QIAcube in the future. Instructions for packing the QIAcube are given in Section 4.5, page 4-7. Using the original package minimizes damage during transportation of the QIAcube.

4.4 Installing the QIAcube

Before operating the QIAcube:

- Remove the QIAcube accessories.
- Carefully peel off the protective film from the QIAcube door.
- Remove the protector for the X- and Y-axes.
- Connect the power cord to the back of the QIAcube.
- Switch on the QIAcube.
- The centrifuge rotor and buckets must be installed.

Remove QIAcube accessories

Remove the QIAcube User Manual, power cord, and the centrifuge rotor and buckets from the form packing material on top of the QIAcube.

Remove the USB stick, rotor key, rotor nut, Allen key, S2 shaker adapter, and shaker rack plugs from the waste drawer.

Removing the protective film from the QIAcube door

Carefully peel off the protective film from the QIAcube door.

Remove the foam protector

During transportation, a foam protector prevents the movable parts of the QIAcube from moving along the X- and Y-axes. Before using the instrument, this protector must be removed.





Foam protector.

Remove the foam protector as follows:

Gently pull the foam protector towards you to remove it (see picture above). After removing the protector for the X- and Y-axes, make sure to close the Q!Acube door.

Installation of AC power cord

- 1. Remove the power cord from the foam packing material on top of the QlAcube. Ensure that the power switch is set to the off position
- 2. Check that the voltage rating on the label at the back of the QIAcube matches the voltage available at the installation site.
- 3. Plug the power cord into the power-cord socket.
- 4. Plug the power cord into a grounded power outlet.

Switching on the QIAcube

Check that the QIAcube operates properly:

- 1. Make sure that the door of the QIAcube is closed.
- 2. Switch on the QIAcube using the power switch. The following events occur in the order shown:
 - The beeper sounds and the startup screen appears.
 - The instrument automatically performs initialization tests.
 - The centrifuge lid opens.



If there is an initialization error, check that the power cord is properly connected to the QIAcube and power outlet. Retry the initialization process. If the problem persists, contact QIAGEN Technical Services.

Note: If you switched on the QIAcube before closing the door, the instrument will not perform an initialization and the centrifuge lid will not open. Switch off the QIAcube, close the door, and then switch on the QIAcube again.

Installing the centrifuge rotor and buckets

The rotor can be mounted in only one orientation. The pin on the rotor shaft fits into a notch on the underside of the rotor directly underneath rotor position 1. Line up position 1 of the rotor with the pin on the rotor shaft and carefully lower the rotor and tighten using the rotor key supplied with the QIAcube. Make sure that the rotor is securely seated. Check that all buckets are properly suspended and can swing freely.

Note: The side of the rotor bucket that must face towards the rotor shaft is marked with a gray line to help prevent buckets from being loaded incorrectly

Important: All centrifuge buckets must be mounted before starting a protocol run, even if fewer than 12 samples are to be processed.

WARNING



Risk of personal injury and material damage [w17]
The roter nut must be securely tightened using the roter key supplied with QIAcube. If the roter nut is not tightened properly, it can become loose during operation of the centrifuge.

Installing the shaker adapter

A shaker adapter must be installed before the shaker can be used. Two types of shaker adapter are available:

- Adapter for 2 ml microcentrifuge safe-lock tubes (marked with "2")
- Adapter for 2 ml screw-cap tubes (marked with "S2").

The QIAcube is delivered with the shaker adapter for 2 ml microcentrifuge safe-lock tubes already installed. If you need to install the shaker adapter for 2 ml screw cap tubes:

- 1. Remove the shaker rack.
- 2. Remove the shaker adapter for 2 ml microcentrifuge safe-lock tubes by unscrewing the retaining screws. Use the Allen key supplied with the QIAcube.
- 3. Place the shaker adapter for 2 ml screw-cap tubes onto the shaker.
- 4. Tighten the 2 retaining screws using the Allen key.

Make sure to use the correct adapter for the sample tubes as this helps to ensure optimal instrument performance. Using an incorrect shaker adapter can adversely affect pipetting performance and protocol results. For more information about sample tubes and the appropriate shaker adapters, see the corresponding protocol sheet.

4.5

Packing the QIAcube

WARNING

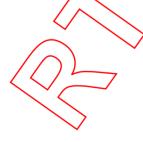
Risk of personal injury and material damage [w2]
The QIAcube is too heavy to be lifted by one person. To
avoid personal injury or damage to the instrument, do not
lift the instrument alone.

If you need to transport the QIAcube, the instrument must first be decontaminated (see Section 6.5) and then prepared as follows:

1. Prepare packing material. Materials required are the cardboard carton, the pallet with foam blocks, the foam lid, and the foam protector for the X- and Y-axes.

The centrifuge lid must be open to allow access to the inside of the centrifuge. If the lid is not open, perform the following steps.

- 1. Close the instrument door.
- 2. Press "Tools" in the main menu.
- 3. Highlight "Maintenance" (scroll through the list by pressing "♠" or "♥"), and press "Select".
- Highlight "Open lid" (scroll through the list by pressing "A" or "▼"), and press "Select".
- 5. Open the instrument door.
- 6. Undo the rotor nut on top of the rotor sing the rotor key and carefully lift the rotor off the rotor shaft
- 7. Close the door.
- 8. Press "Tools" in the main menu
- Select "Maintenance" by pressing "A" or "▼" to scroll through the list until it is highlighted, and then press "Select".
- 10. Select the protocol "Close lid" by pressing "▲" or "▼" to scroll through the list until it is highlighted, and then press "Start".
- 11. When the centrifuge lid is closed, switch off the QIAcube and open the door.
- 12. Insert the foam protector into the front of the instrument.
- 13. Press the foam down between the centrifuge and the robotic arm.





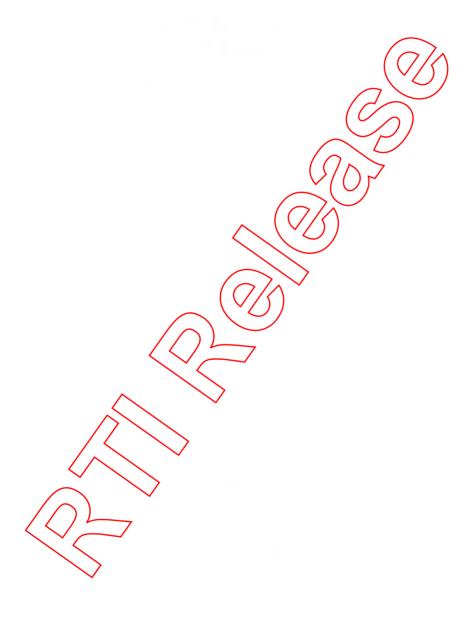
Foam protector inserted between the centrifuge and the robotic arm.

- 14. Push the foam until the rear end touches the back wall of the instrument. Ensure that the arm is held firmly in place and cannot move.
- 15. Make sure that the CTAsube door can be closed properly. The door should lightly brush against the foam.
- 16. Place the accessories into the drawer. The following accessories should be packed in air cushion bags:
 - Rotor key-
 - Rotor nut
 - USB stick
 - Allan key
 - Shaker rack plugs
 - Shaker adapter
- 7. Place the QIAcube onto the pallet and put the black foam lid over the top of the instrument. Place the box onto the instrument. When lifting the QIAcube, slide your fingers under both sides of the workstation and keep your back straight.
- 18. Place accessories into the black foam lid. The following accessories should be packed in air cushion bags:
 - Rotor with swing-out buckets
 - Power cords
 - QIAcube User Manual



19. Seal the outside edges of the carton with tape to protect against moisture.

Note: Using the original package minimizes damage during transportation of the QlAcube.



5 Operating Procedures

This section describes how to operate the QIAcube.

Before proceeding, we recommend that you familiarize yourself with the features of the instrument by referring to Section 3.

important: The QIAcube is designed for use with QIAGEN spin columns only. Geometry of spin columns manufactured by other suppliers may not be compatible with the QIAcube.

CAUTION



Damage to the instrument

Only use QIAGEN spin columns and QIAGEN Spin Kits with the QIAcube. Damage caused by use of other types of spin column or chemistries will voic your warranty.

The door of the QIAcube must remain closed during operation of the instrument. Only open the door when instructed to do so by the software.

WARNING



Risk of personal injury and material damage

Do not attempt to move the QIAcube during operation.

WARNING



Moving parts

[W11

[W3]

To avoid comact with moving parts during operation of the QIAcube, the instrument must be operated with the door closed.

If the door sensor is not functioning correctly, contact QIAGEN Technical Services.



5.1 QIAcube software

The QlAcube is operated through the touchscreen. The functions of some of the buttons are described below.

Enables the user to scroll up through a list.

Enables the user to scroll down through a list.

Select Enables the highlighted parameter to be

selected.

Back Returns to the previous step

Options Lists the available options for the protocol

(e.g., "Remove").

Cancel Cancels the previous command or returns to

the previous step without saving any changes.

Edit Enables the user to enter values for certain

parameters (e.g., elution volume).

+ Increases the displayed value.

Decreases the displayed value.

Default Enables the default value of the highlighted

parameter to be used.

Save Enables changes to be saved to the protocol.

Starts a protocol run.

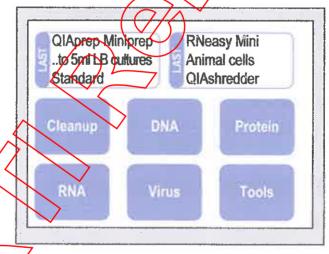
5.2 Running a QIAGEN protocol

The most popular QIAGEN standard protocols are already installed on the QIAcube. All available QIAGEN protocols, including the corresponding protocol sheets, can be downloaded from the QIAcube web portal (www.qiagen.com/MyQIAcube). Refer to the handbook supplied with the QIAGEN kit you are using and the relevant

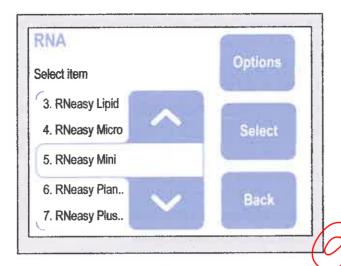
protocol sheet to find out which protocol to load and to obtain further details about the protocol.

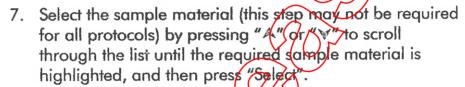
5.2.1 Starting a protocol

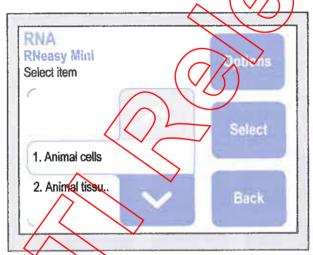
- Carefully read the handbook supplied with the QIAGEN kit and the relevant protocol sheet (available at www.qiagen.com/MyQIAcube) before starting.
- 2. Prepare the samples and reagents to be used in the protocol. Refer to the handbook supplied with the kit and to the relevant protocol sheet for instructions.
- 3. Close the instrument door.
- 4. Switch on the QIAcube at the power switch.
 - The beeper sounds and the startup screen appears.
 - The instrument automatically performs initialization tests.
- 5. Select the appropriate application from the main menu by pressing "Cleanup", "DNA", "Protein", "RNA", or "Virus".



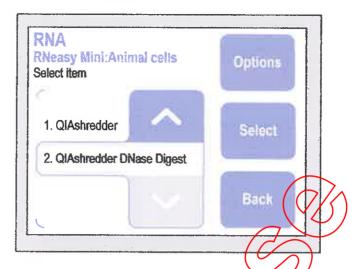
Select the correct kit name by pressing "A" or "\v" to scroll through the list until the correct kit name is highlighted, and then press "Select".



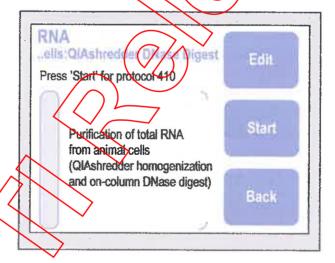




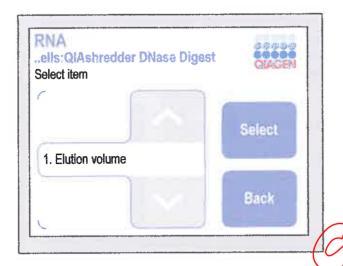
Bepending on the protocol, further options may be available (e.g., standard protocol, homogenization using a QIAshredder column, on-column DNase digest, etc.). Select the protocol by pressing "A" or "V" to scroll through the list until the required protocol is highlighted, then press "Select"...



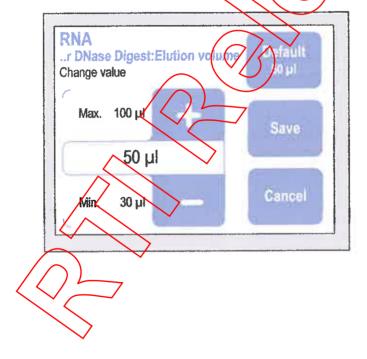
 To start the protocol run, press "Start" and proceed to step 12. To enter user-defined values for certain parameters such as elution volume, press "Edit".
 Note: Not all protocols allow user-defined values to be entered.

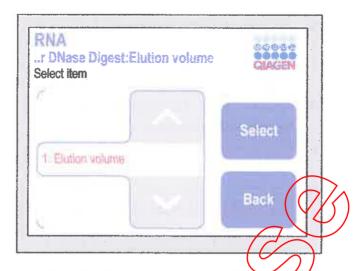


10. Press "A" or "∀" to highlight the parameter to be edited, for example, "Elution volume", and then press "Select".

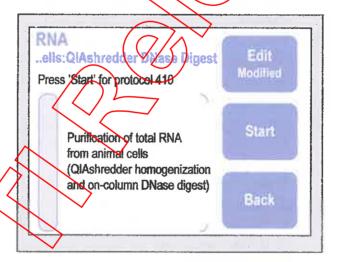


11. Change the elution volume by pressing "+" or "-". The default value is displayed in the "Default" button. Press "Save" to save the changes. The parameter that has been changed will then be displayed in red. Press "Back" to exit edit mode.





12. The "Edit" button now indicates that the protocol has been modified. If run reports should be automatically saved to the USB stick, insert the USB stick into the USB port (see Section 5.5). Press "Start" to start the protocol run.



- 13. Open the worktable door.
- 14. Follow the instructions in the protocol sheet and displayed in the touchscreen for loading the worktable with samples, reagents, and labware (see Section 5.3 for more information about worktable setup). You will be instructed to:
 - Empty the waste drawer.
 - Place prefilled tip racks onto the worktable.

- Fill the reagent bottles and place the reagent bottle rack onto the worktable. Be sure to remove the lids from the reagent bottles before placing on the worktable.
- Load opened accessory-buffer tubes required by the protocol in positions A, B, or C.
- Place the loaded rotor adapters into the centrifuge buckets.
- Load the shaker rack with samples, and place onto the shaker. Be sure to use the correct sample tubes and the correct shaker adapter, as described in the protocol sheet (see page 5-16).
- 15. Close the QlAcube door.
- 16. After you have set up the worktable, the QIAcube performs a load check, which consists of the following tasks:
 - The instrument is initialized.
 - The shaker is checked to make sure it is correctly loaded.
 - The number of rotor adapters in the centrifuge is checked to make sure that it corresponds to the number of samples in the shaker and that the rotor is correctly loaded.
 - The reagent bottle rack is checked to make sure that it is correctly loaded and there is sufficient buffer in the reagent bottles.

Note: The volume of accessory buffers in positions A, B, or C on the worktable is not checked. Be sure to fill the microcentrifuge tubes with the exact volume of buffer required.

- The filter-tips are checked to make sure that the correct type has been loaded and that there are sufficient filter-tips for the protocol run.
- 17. If the load check is unsuccessful, an error message will be displayed in the touchscreen. Resolve the error so that the protocol run can continue (see Section 7.2.1).

Note: When resolving a load check error, only move or change the affected samples or consumables.

5.2.2 End of the protocol run

When the protocol run has finished, a message is displayed in the touchscreen confirming that the samples have been processed. Follow the instructions in the touchscreen for worktable cleanup.

- 1. Remove the microcentrifuge tubes containing purified nucleic acids or proteins from the rotor adapters.
- 2. Discard sample tubes, used rotor adapters, and reagents according to your local safety regulations.
- 3. Replace the lids of the reagent bottles and close tightly. Store the bottles according to the instructions in the relevant kit handbook.
- 4. Empty the waste drawer.
- 5. Run another protocol, or switch off the QIAcube.

5.2.3 Stopping a protocol

You can stop a QIAGEN protocol if there is an emergency by pressing "Cancel". To confirm that you want to stop the protocol run, press "OK". To cancel the stop protocol command, press "Cancel".

Note. If a protocol run is stopped, the run cannot be restarted; the samples must be processed manually (see Sections 7.2.0 and 7.2.7).

5.3 Setting up the worktable

5.3.1 Loading the reagent bottle rack

Attaching labeling strips to the reagent bottle rack

Two rack labeling strips must be attached to the reagent bottle rack. These strips are kit-specific and identify the positions into which the QIAGEN buffers required for the protocol should be placed. In addition, the strips ensure that the reagent bottle rack is positioned correctly on the worktable for liquid detection during the load check.

Attach each rack labeling strip by sliding it onto the flange at each side of the rack. The labeling strips fit onto the rack only in the correct orientation.

Note: The reagent bottle rack must only be used with the labeling strips attached.



Aftaching labeling strips.

Carefully fill each bottle with buffer, and place into the appropriate position in the reagent bottle rack indicated by the rack labeling strip. Pour carefully to ensure that the buffer does not foam or contain large air bubbles. Reagent volumes are checked at during the load check at the start of the protocol fun.

important: Be sure to remove lids from the bottles before plaging onto the worktable.

When instructed to do so, place the reagent bottle rack containing buffer bottles onto the worktable. The rack fits onto the worktable only in the correct orientation.

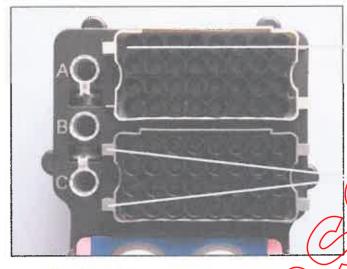
into the designated slot of the worktable. Leaning positioned bottle racks could lead to errors during liquid detection.



Loading the reagent bottle razk.

5.3.2 Loading the tip racks

The QlAcube is provided with 3 tip racks: a rack for $200 \,\mu$ l filter-tips (blue-colored), a rack for $1000 \,\mu$ l filter-tips (light-gray colored), and a rack for $1000 \,\mu$ l wide-bore filter-tips (dark-gray colored). Each tip rack has a specially positioned notch that the optical sensor uses to identify the tip rack during the load check. The rack for $200 \,\mu$ l filter-tips has a notch at the left forward side, the rack for $1000 \,\mu$ l filter-tips has a notch at the left rear side, and the rack for $1000 \,\mu$ l wide-bore filter-tips has 2 notches. During the load check, the QlAcube checks that the correct tip racks have been placed on the worktable and that there are sufficient tips for the protocol run. When refilling tip racks, make sure to load the racks with the correct size of filter-tip. Only use filter-tips designed for use with the QlAcube.



Tip rack for $1000 \,\mu l$ filtertips has 1 notch at the left rear side

Tip rack for 1000 µl widebore filter-tips was 2 norches

QIAcube tip racks.

Important: Do not use damaged filter-tips: Do not load damaged tip racks onto the worktable.

5.3.3 Loading the rotor adapters

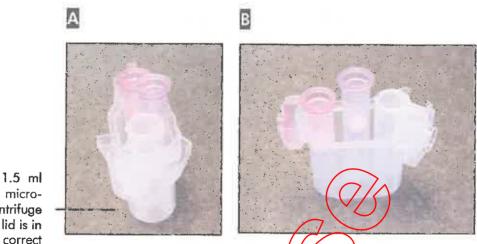
Rotor adapters can be placed into the rotor adapter holder enabling convenient and easy loading. Place 1.5 ml microcentrifuge tubes (supplied with the rotor adapters) and QIAGEN spin columns into the appropriate positions in each rotor adapter as described in the corresponding protocol sheet. Ensure the 1.5 ml microcentrifuge tubes and spin columns have been pushed firmly into the appropriate position.

WARNING



Risk of personal injury and material damage

Make sure that lids from spin columns and 1.5 ml
microcentrifuge tubes are in the correct position and
pushed all the way down to the bottom of the slots on the
sides of the of the rotor adapter. Incorrectly positioned lids
can break off during centrifugation.



microcentrifuge tube lid is in correct

> Correctly loaded rator adapter, A Retor adapter is correctly loaded and the 1.5 ml microcentrifuge tube lick is in the correct position; Correctly loaded rotor adapter seen from the side.



Insorrectly loaded rotor adapter. A Rotor adapter is incorrectly loaded with a 1.5 ml microcentrifuge tube. The lid of the tube is not pushed all the way down to the bottom of the slot of the rotor adapter and could break off during centrifugation, causing the protocol run to crash (compare with part A of the previous figure); B Incorrectly loaded rotor adapter seen from the side (compare with part 3 of the previous figure).



1.5 ml microcentrifuge tube lid is in the wrong slot of the rotor adapter

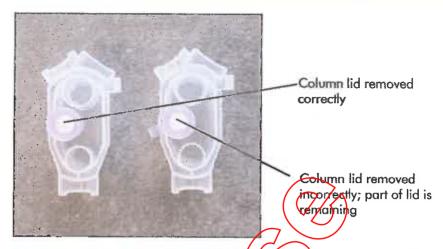
Rotor adapter is incorrectly loaded with a 1.5 ml pricrocentrifuge tube. The lid of the tube is positioned in the wrong stat of the rotor adapter. During column transfer the lid of the spin column could crash onto the lid of the 1.5 ml microcentrifuge tube, causing the protocol run to crash.

If the protocol requires the use of a column in the middle position (e.g., QlAshredder spin column), cut off the lid before loading the column.

WARNING



Risk of personal injury and material damage [W19]
Be sure the hid is completely removed from the spin column. Spin columns with partially removed lids may not be removed properly from the rotor, causing the protocol run to spash.



Loading a column into the middle pesition.

If the protocol requires use of spin columns with screw caps (e.g., Qproteome Albumin/IgG/Deptetion Spin Columns), remove the screw-cap from the spin column, and screw a spin column adapter ring (not supplied with the QIAcube; cat. no. 990399) onto the column.



Break off the bottom closure of the spin column, and place the spin column into the appropriate position of the rotor adapter.

The spin column adapter ring enables the robotic gripper to transfer the column during the purification procedure.

Place the loaded rotor adapters into the centrifuge buckets when instructed to do so by the software. For ease of use and high process safety, the rotor adapters only fit into the centrifuge buckets in one orientation.

Note: If processing fewer than 12 samples, make sure to load the centrifuge rotor symmetrically. For more information, see Appendix B or the loading chart available at www.qiagen.com/MyQlAcube. All centrifuge buckets must be mounted before starting a protocol run, even if fewer than 12 samples are to be processed.

WARNING



Risk of personal injury and material damage [W16]
Do not use damaged rotor adapters. The rotor adapters are for single use only. Do not reuse the rotor adapters as they can be damaged by the high or forces experienced in the centrifuge.



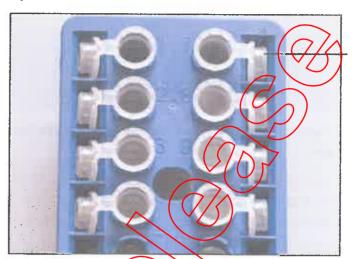
Looding the centrifuge.

5.3.4 Loading the samples

Make sure that the correct shaker adapter is installed (see Section 4, page 4-6). Two types of shaker adapter are available:

- Adapter for 2 ml safe-lock microcentrifuge tubes (marked with "2")
- Adapter for 2 ml screw-cap tubes (marked with "S2")

For more information about the required sample tube types (e.g., Sample Tubes RB (2 ml) cat. 990381, Sample Tubes CB (2 ml) cat 990382), see the relevant protocol sheet. Load opened sample tubes into the shaker rack. The sample positions are numbered for ease of loading. Place the loaded shaker rack onto the shaker when instructed to do so by the software.



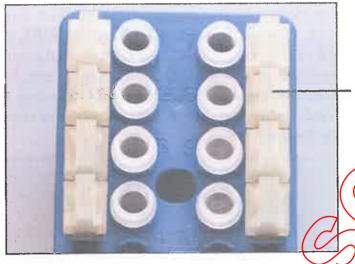
Lids of sample tubes must be securely placed into slots at the edge of the shaker rack

Loading the shaker rock with samples tubes that have attached lids.

If using 2 ml safe-lock microcentrifuge tubes, place the lids of the tubes into the slots at the edge of the shaker rack.

Important: Make sure that the lids are securely seated in the stats.





Loading the shaker rack with samples tobes that have screw caps.

If using 2 ml screw-cap tubes, place the shaker rack plugs into the slots at the edge of the shaker rack. This enables detection of samples during the load check.

Note: If processing fewer than 12 samples, make sure that the shaker is correctly loaded. For more information, see Appendix B or the loading chart available at www.giagen.com/MyQlAsube.

WARNING

Risk of material damage

[W20]

Shaker rack plugs must be placed into slots at the edge of the chaker rack

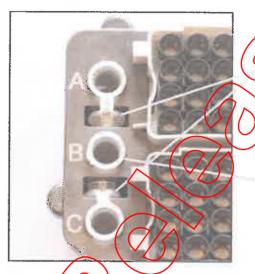


Do not use 1.5 ml microcentrifuge tubes on the shaker. Because 1.5 ml microcentrifuge tubes can cause filter-tips to stick during sample transfer, their use on the shaker can darnage the pipetting system and can cause a centrifuge crash.

5.3.5 Loading accessory buffers

Up to three 1.5 ml or 2 ml microcentrifuge tubes containing accessory buffers can be placed onto the QIAcube worktable. The exact volume of buffer required for the protocol depends on the number of samples. Buffer volume, tube type, and the position into which the tube should be placed are described in the relevant protocol sheet (available at www.qiagen.com/MyQIAcube).

check on these positions, it is absolutely necessary to use the correct tube type filled with exactly the volume of buffer described in the protocol sheet. Incorrect buffer volumes can lead to pipetting errors during the protocol run. Make sure to open the tubes before placing them onto the worktable. Lids of microcentrifuge tubes should be securely placed into the slots at positions A and C. If 3 tubes are to be placed onto the QIAcube worktable, the lid of 1 tube must be cut off.



Lids of 2 of the microcentrifuge tubes must be securely placed in slots at positions A and C.

The lid of the third tube must be cut off.

Correct leading of accessory buffers.



The lid of the third tube has not been cut off, which may lead to a collision with the tip adapter.

incorrect loading of accessory buffers.

5.4 Installing and deleting protocols

At delivery the most common standard QIAGEN protocols are already installed on your QIAcube. The range of protocols available is continually expanding, and additional standard QIAGEN protocols can be downloaded free of charge at www.qiagen.com/MyQIAcube. In addition, our highly qualified Application Specialists can customize QIAGEN protocols or develop new protocols tailored to your needs. Protocols that are no longer required can be easily removed from the QIAcube.

Installing new protocols (standard QTAGEN protocols)

New protocols for the QIAcube can be downloaded at www.qiagen.com/MyQIAcube. To transfer new protocols to the QIAcube, first save them to the USB stick.

- 1. Create a folder named "New Protocols" on the USB stick.
- 2. Go to www.qiagen.com/MyQIAcube and search for the required protocol.
- 3. Select the protocol to be downloaded and save it to the "New_Protocols" tolder on the USB stick.
- 4. Make sure that the QIAcube is switched on.
- 5. To install the protocol, connect the USB stick to the QIAcube via the USB port, which is located at the front right of the instrument.
- 6. In the main menu, press "Tools".
- 7. Select "Data Exchange" by pressing "▲" or "▼" to scroll through the list until it is highlighted, and then press "Select".
- 8. To transfer protocols to the QIAcube, select "Protocols" and either the "Load individually from USB" or the "Load all from USB" option.
 - Load individually from USB: Select the required protocol by pressing "▲" or "▼" to scroll through the list until it is highlighted, and then press "Select". Start the download procedure by pressing "OK". A message will confirm that

the protocols have been successfully transferred to the QIAcube.

Load all from USB: Select "Load all protocols" and confirm with "OK". All files of the .qpf format will be transferred from the USB stick to the QIAcube. A message will confirm how many protocols have been successfully transferred to the QIAcube.

Note: Protocols already installed on the QIAcube that have the same name as downloaded protocols will be overwritten.

Note: A maximum of 48 protogols can be transferred to the QIAcube.

When the protocols have been successfully transferred to the QIAcube, press "OK"

Installing new protocols (customized QIAGEN protocols or customer-specific protocols)

Customized QIAGEN protocols or new protocols developed to meet your specific requirements can be purchased from QIAGEN.

- 1. Go to www.qicaen.com/MyQlAcube and enter the required information. If our Application Specialists require additional information, they will contact you directly.
- 2. You will be informed by e-mail when your customized protocol is ready for download.
- Click the link in the e-mail to go to the download page.
- 4. To install the protocol on your QIAcube, follow steps 3–8 in "Installing new protocols (standard QIAGEN protocols)", page 5-20.

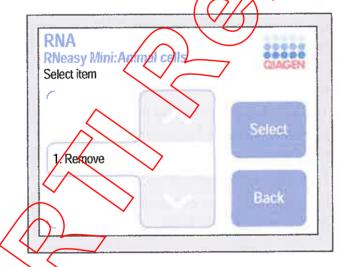


Deleting protocols

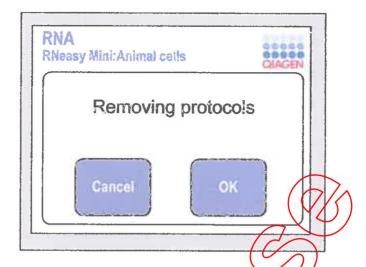
Protocols can individually be removed from the QIAcube by deleting the protocol type or they can be removed in packages by deleting the kit name or starting material.

To remove a single protocol:

- 1. Select the required application from the main menu.
- 2. Select the correct kit name by pressing "A" or "V" to scroll through the list until the correct kit name is highlighted, then press "Select".
- 3. Select the starting material (this step may not be required for all protocols) by pressing "A" or "V" to scroll through the list until the correct starting material is highlighted, and then press "Select",
- 4. Select the protocol type by pressing "or "v" to scroll through the list until the correct protocol is highlighted, and then press "Options".
- 5. To remove the protocol, select "Remove", and then press "Select".



6. Press "OK" to remove the protocol.



To remove a protocol package:

- 1. Select the required application from the main menu.
- 2. Select the correct kit name by pressing "A" or "\" to scroll through the list until the correct kit name is highlighted, then press "Options".
- 3. To remove all protocols of the selected kit, select "Remove", and then press "Select".
- 4. Press "OK" to tempve the protocol.

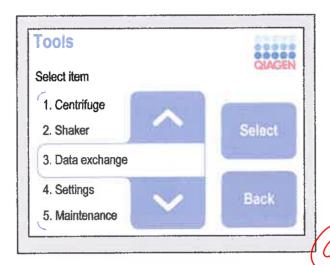
5.5 Saving data files

Data files, such as run reports, log files, error logs, or statistics, can be saved to the USB stick.

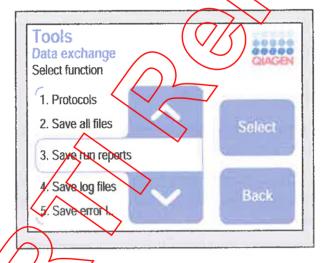
Note: The QIAcube can store up to 9 log files and run reports. After the tenth protocol run, the oldest log file and run report will be overwritten.

- 1. Make sure that the QIAcube is switched on.
- 27 Connect the USB stick to the QIAcube via the USB port, which is located at the front right of the instrument.
- 3. In the main menu, press "Tools".
- 4. Select "Data exchange" by pressing "A" or "▼" to scroll through the list until it is highlighted, and then press "Select".

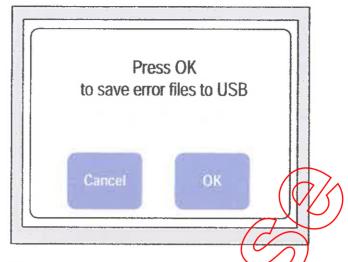
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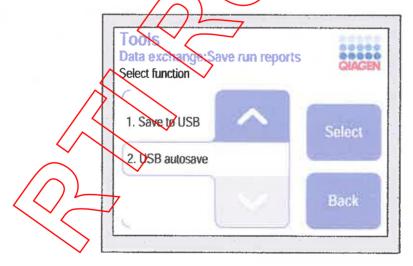
5. Select the appropriate type of data file (e.g., "Save run reports", "Save log files", "Save error logs", "Save statistics", or "Save calibration") by pressing "♠" or "♥" to scroll through the list until the correct data file is highlighted, and then press "Select". To save all files, select "Save all files".



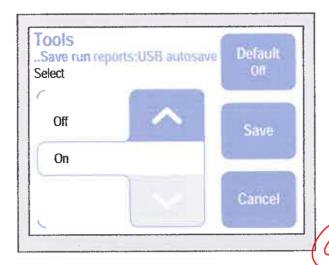
o. Press "OK" to save the data file to the USB stick.



- 7. A message will appear to confirm that the data file has been successfully saved to the USB stick.
- 8. When the data file has been successfully saved to the USB stick, press "OK"?
- 9. For run reports only, an additional option is available for automatically saving the run report to the USB stick at the end of each protocol run. Select "USB autosave" by pressing " or " to scroll through the list until it is highlighted, and then press "Select".



10. Select "Off" or "On" by pressing "A" or "♥" to scroll through the list until the appropriate option is highlighted. The default value is displayed in the "Default" button. Press "Save" to save the changes.

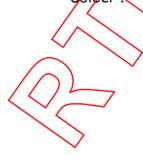


5.6 General information

System information

System information, such as the instrument serial number, the number of installed protocols, as well as the system's memory capacity (maximum and currently available), can be easily displayed.

- 1. In the main menu, press Tools".
- 2. Select "Service" by pressing "♠" or "♥" to scroll through the list until it is highlighted, and then press "Select".
- 3. Select "System info" by pressing "♠" or "♥" to scroll through the list until it is highlighted, and then press "Select".



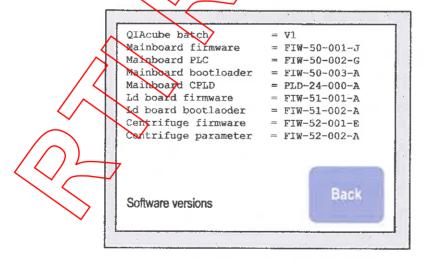
```
QIAcube batch = V1
Serial number = 4376
Number of protocols = 16
Max. Flash space = 1690 kB
Free Flash space = 1132 kB

System Info
```

Identification of software versions

The software versions that are installed on your QlAcube can be easily identified.

- 1. In the main menu press "Tools".
- 2. Select "Service" by pressing "♠" or "♥" to scroll through the list until it is highlighted, and then press "Select".
- 3. Select "Software versions" by pressing "♠" or "♥" to scroll through the list until it is highlighted, and then press "Select".



 Compare the installed software versions with the newest available on the QlAcube Web Portal (available at www.qiagen.com/MyQlAcube). We recommend always

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installing the latest software version to ensure the best performance of your instrument. The software versions can be distinguished by the alphabetic index at the end of the identifier of the software versions.

Software update

For detailed information on how to upgrade the software versions of your QIAcube refer to the software update documentation on the QIAcube Web Portal (available at www.qiagen.com/MyQIAcube).

Display brightness

The brightness of the QIAcube touchscreen can be adjusted.

- 1. In the main menu, press "Tools" /
- 2. Select "Settings" by pressing "A" or "" to scroll through the list until it is highlighted, and then press "Select".
- 3. Select "Brightness" by pressing "✓" or "✓" to scroll through the list until it is highlighted, and then press "Select".
- 4. The brightness of the touchscreen can be adjusted by pressing "+" or "-".
- 5. To save the change, press "Save". To exit without saving the change, press "Cancel".

Beeper volume

- 1. In the main menu, press "Tools".
- 2. Select "Settings" by pressing "♠" or "▼" to scroll through the list until it is highlighted, and then press "Select".
- Select "Beeper volume" by pressing "▲" or "▼" to scroll through the list until it is highlighted, and then press "Select".
- 4. The volume of the beeper can be adjusted by pressing "+" or "-".
- 5. To save the change, press "Save". To exit without saving the change, press "Cancel".

Maintenance

To reinitialize the QIAcube, to open or close the centrifuge lid, to perform a tightness test, or to gain access to the robotic arm for cleaning, follow the instructions below.

- 1. In the main menu, press "Tools".
- 2. Select "Maintenance" by pressing "♠" or "♥" to scroll through the list until it is highlighted, and then press "Select".
- 3. Select "Initialize", "Open lid", "Cleaning position", or "Tightness test",

5.7 Operating the shaker

The shaker can be operated individually if the QIAcube is not performing a protocol run. The shaker can be used to heat and shake samples simultaneously, shake samples without heating, or heat samples without shaking.

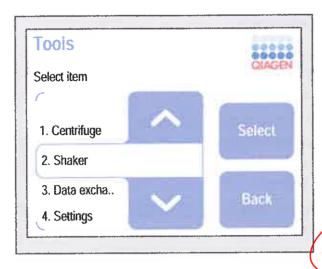
WARNING

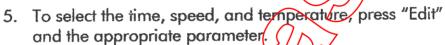


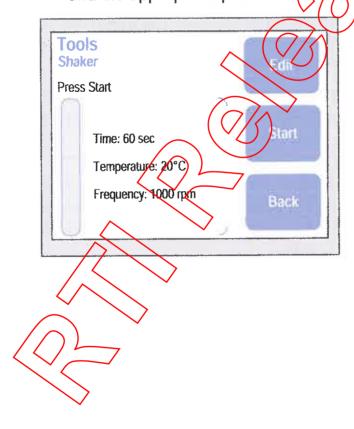
Risk of personal injury and material damage [w3]
Do not attempt to move the QIAcube during operation.

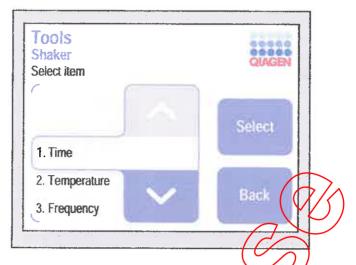
- 1. Switch on the QIAcube at the power switch.
 - The beeper sounds and the startup screen appears.
 - The instrument automatically performs initialization tests.
- 2. Open the QIAcube door.
- 3. In the main menu, press "Tools".
- Select "Shaker" by pressing "♠" or "∀" to scroll through the list until it is highlighted, and then press "Select".



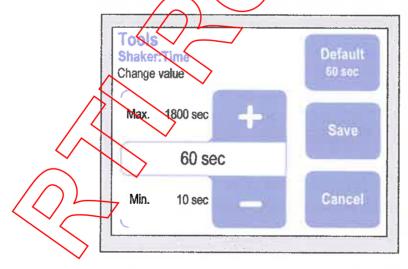






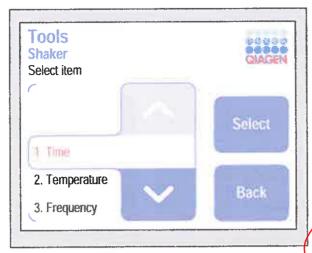


- 6. The values for each parameter can be changed by pressing "+" or "-", and can be set within the ranges given below. The default value is displayed in the "Default" button. Press "Save" to save the changes.
 - Time: 10-1800 seconds, in increments of 10 seconds
 - Temperature: ambient temperature to 70°C, in increments of 1°C
 - Frequency 0-2000 rpm, in increments of 100 rpm



If you have changed a parameter, it will be highlighted in red color.

7 Press "Back" to exit edit mode.





9. Press "Start" to start the shaker.

5.8 Operating the centrifuge

The centrifuge can be operated individually if the QIAcube is not performing a protocol run.

WARNING



Risk of personal injury and material damage [w3]

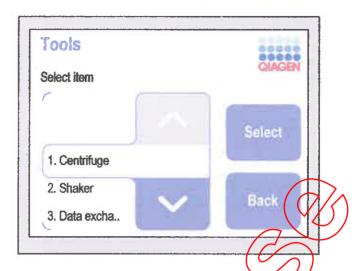
Do not attempt to move the QIAcube during operation.

Switch on the QlAcube at the power switch.
 The beeper sounds, and the startup screen appears.
 The instrument automatically performs initialization tests.

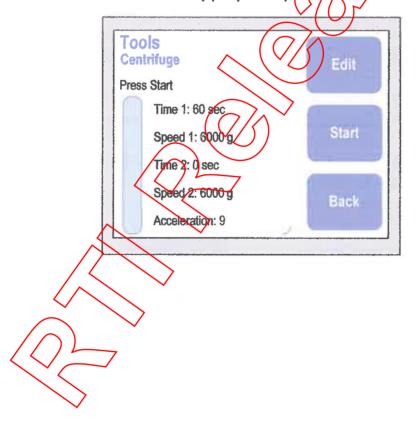
2. Open the QlAcube door.

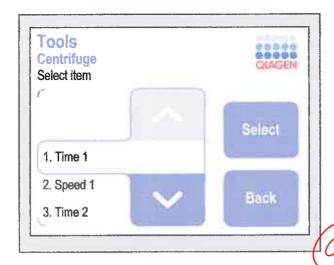
3/ In the main menu, press "Tools".

Select "Centrifuge" by pressing "A" or "∀" to scroll through the list until it is highlighted, and then press "Select".



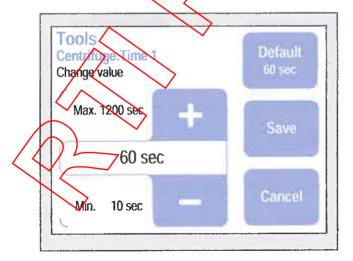
5. To select the speed, time, and acceleration, press "Edit" and the appropriate parameter.







- 6. The values for each parameter can be charged by pressing "+" or "-", and can be set within the ranges given below. The default value is displayed in the "Default" button. Press "Save" to save the changes.
 - Time 1: 10–1200 seconds in increments of 10 seconds
 - Speed 1: 1000–12,000 x g, in increments of 100 x g
 - Time 2: 0–1200 seconds, in increments of 10 seconds
 - Speed 2: 1000-12,000 x g, in increments of 100 x g
 - Acceleration: 1-9

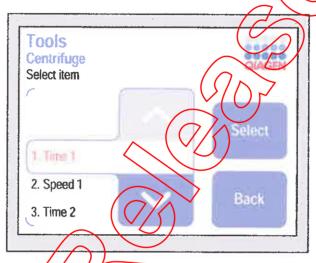


To run the centrifuge for a specified length of time at one speed, enter values for "Time 1" and "Speed 1" and enter 0 for "Time 2".

If required, the centrifuge can be set to run for a specified time at a given speed ("Time 1" and "Speed 1") and then can run at an increased or decreased speed for a specified time ("Time 2" and "Speed 2").

The acceleration rate of the centrifuge can be set by entering a value for "Acceleration". A value of 1 gives the slowest rate of acceleration; a value of 9 gives the maximum rate of acceleration.

7. If you have changed a parameter, it will be highlighted in red color. Press "Back" to exit the edit mode.



8. If required, load opened 1.5 ml microcentrifuge tubes and/or QIAGEN spin columns into the rotor adapters and place the lids into the appropriate slots in the rotor adapter. Place the rotor adapters into the centrifuge, and close the QIAcube door. For more information about loading the rotor adapters, see Section 5.3.3.

load the centrifuge rotor symmetrically. For more information, see Appendix B or the loading chart available at www.qiagen.com/MyQIAcube. All centrifuge buckets must be mounted, even if fewer than 12 samples are to be processed.

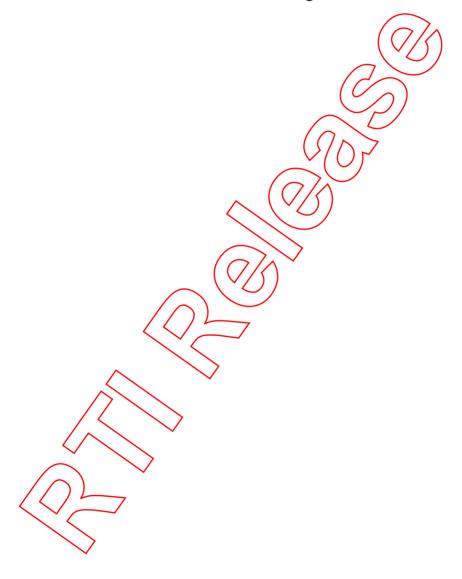


WARNING



Risk of personal injury and material damage [W16] Do not use damaged rotor adapters. The rotor adapters are for single use only. Do not reuse the rotor adapters as they can be damaged by the high g forces experienced in the centrifuge.

9. Press "Start" to start the centrifuge.



6 Maintenance Procedures

The following maintenance procedures must be carried out to ensure reliable operation of the QIAcube:

- Regular maintenance after each protocol run
- Daily maintenance after the last protocol run of the day
- Monthly maintenance every month
- Periodic maintenance when necessary; at least every 6 months

Following these procedures ensures that the QIAcube is free of dust and liquid spills.

The cleaning agent is chosen according to the goal of the cleaning procedure, the sample material, and the downstream assay.

Cleaning agents

The following disinfectants and detergents are recommended for cleaning the QlAcube.

Note: If you want to use disinfectants different from those recommended, ensure that their compositions are similar to those described below.

General cleaning of the QIAcube:

- Mild Detergents
 - 70% ethanol



Disinfection

- Ethanol-based disinfectants can be used for disinfection of surfaces, such as the worktable or inside the centrifuge: e.g., 25 g ethanol and 35 g 1-propanol per 100 g liquid or Mikrozid® Liquid (Schülke & Mayr GmbH, cat. no. 109160).
- Disinfectants based on glyoxal and quaternary ammonium salt can be used for submerging worktable items, the centrifuge rotor, and the waste drawer; e.g., 10 g glyoxal, 12 g lauryldimethylbenzylammonium chloride, 12 g myristyldimethylbenzylammonium chloride, and 5–15% nonionic detergent per 100 g liquid, Lysetol® AF (Gigasept® Instru AE in Europe, cat. no. 107410, or DECON-QUAT® 100, Veltek Associates, Inc., in the USA, cat. no. DQ100 06-167-01)

Removal of RNase contamination

RNaseZap® RNase Decontamination Solution (Ambion, Inc., cat. no AM9780) can be used for cleaning surfaces and submerging worktable items, centrifuge rotor, and waste drawer.

Removal of nucleic acid contamination

DNA-Exitus Plus (AppliChem, cat. no. A7089,0100) can be used for cleaning surfaces and submerge worktable items, centrituge rotor, and waste drawer.

General instructions

- Do not use spray bottles to spray cleaning or disinfectant liquids onto surfaces of the QIAcube workstation. Spray bottles should be used only for items that have been removed from the workstation.
- If solvents or saline, acidic, or alkaline solutions are spilt on the QIAcube or if QIAGEN buffers splash the instrument door, wipe the spilt liquid away immediately.
- Follow manufacturer's safety instruction for handling cleaning agents.
- Follow manufacturer's instruction for soaking time and concentration of the cleaning agents. Immersing for longer than the recommended soak time can harm the instrument.

- Do not use alcohol or alcohol-based disinfectants to clean the QIAcube door. Exposing the QIAcube door to alcohol or alcohol-based disinfectants will cause surface crackina. Clean the QlAcube door with distilled water only.
- Do not submerge buffer bottles in 70% alcohol. The blue ring is not ethanol resistant.
- Take care that no liquid runs down the touchscreen. Liquid may be drawn through the dust protection sealing by capillary forces and cause malfunction of the display. To clean the touchscreen, moisten a soft lint-free cloth with water, ethanol, or a mild detergent and carefully wipe the display. Wipe dry with a poper towel.

CAUTION



Damage to the instrument

[C5] Do not use bleach, solvents, or reagents containing acids, alkalis, or abrasives to clean the QIAcube.

WARNING



Risk of electric shock

Do not open gry panels on the QIAcube.

Risk of personal injury and material damage Only perform maintenance that is specifically described in this user manual

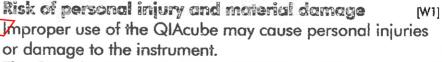
WARNING



Hazardous chemicals and infectious agents [W22] The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed of properly. Refer to your local safety regulations for

proper disposal procedures.

WARMING



The QIAcube must only be operated by qualified personnel who have been appropriately trained.

Servicing of the QIAcube must only be performed by QIAGEN Field Service Specialists.

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W211

WARNING



Risk of fire

W91

When cleaning the QIAcube with alcohol-based disinfectant, leave the QIAcube door open to allow flammable vapors to disperse.

Only clean the QIAcube when worktable components have cooled down.

Servicing

Contact your local QIAGEN Field Service Specialist or your local distributor for more information about flexible Service Support Agreements from QIAGEN.

6.1 Regular maintenance procedure

After running a protocol, perform the regular maintenance procedure:

- 1. Empty the waste drawers
- 2. Remove used disposable labware and unwanted samples and reagents from the worktable. Discard them according to your local safety regulations.
- 3. Replace the lids of the reagent bottles and close tightly. Store the bottles according to the instructions in the relevant kit handbook.

You can now ran another protocol or switch off the QIAcube.

6.2 Daily maintenance procedure

After running the last protocol of the day, perform the daily maintenance procedure:

- 1. Remove used disposable labware and unwanted samples and reagents from the worktable. Discard them according to your local safety regulations.
- 2. Close the buffer bottles tightly and store according to the instructions in the relevant kit handbook.
- 3. Empty the waste drawer and check that the liner is clean. If necessary, clean it with cleaning agent, and then rinse with distilled water.

6.3 Monthly maintenance procedure

Perform the regular maintenance procedure before you perform the monthly maintenance procedure. Select the appropriate cleaning agent according to the sample material and downstream assay (see Section 6).

- 1. Clean the optical sensor, tip adapter, gripper unit, including the gripper, the stabilizing rod, and the spin column lid holder, by carefully wiping these modules with a soft lint-free cloth moistened with water. To gain access to the modules within the robotic arm:
 - Press "Tools" in the main menu.
 - Select "Maintenance" by pressing "A" or "▼" to scroll through the list until it is highlighted, and then press "Select".
 - Select "Cleaning position" by pressing "A" or "\" to scroll through the list until it is highlighted, and then press "Start".
 - Follow the instructions displayed in the touchscreen. You will be instructed to remove the waste drawer and the commune tray.

The robotic arm will move forward and downwards, enabling the modules to be accessed for cleaning through the opening for the waste drawer.



Cleaning modules.

- 2. After cleaning the modules of the robotic arm, switch off the QIAcube at the power switch.
- 3. Thoroughly wipe the worktable with a soft lint-free cloth moistened with cleaning agent. Incubate as appropriate, rinse with distilled water, and wipe dry with paper towels.
- 4. Clean the shaker rack, labware tray, heating adapter, and reagent bottle rack with cleaning agent. Incubate as appropriate, rinse thoroughly with distilled water, and wipe dry with paper towels.
- 5. Clean the liner of the waste drawer with cleaning agent. Incubate as appropriate, rinse with distilled water, and wipe dry with paper towels.
- 6. Thoroughly wipe the inside and outside of the QlAcube using the cleaning agents.

 Important: Do not use alcohol or alcohol-based disinfectants to decontaminate the QlAcube door.

 Important: Take care that no liquid runs down the touchscreen. Liquid may be drawn through the dust protection sealing by capillary forces and cause malfunction of the display. To clean the touchscreen, moisten a soft lint-free cloth with 70% ethanol or a mild disinfectant and carefully wipe the display. Wipe dry with a paper towel.

6.4 Periodic maintenance procedure

Select the appropriate cleaning agent according to the sample material and downstream assay (see Section 6).

6.4.1 Cleaning the centrifuge

The centrifuge lid must be open to allow access to the inside of the centrifuge. The lid should be opened only after the centrifuge has come to a complete stop. If the lid does not open automatically, perform the following steps.

- 1. Press "Tools" in the main menu.
- Highlight "Maintenance" (scroll through the list by pressing "▲" or "▼"), and press "Select".

3. Highlight "Open lid" (scroll through the list by pressing "♠" or "♥"), and press "Select".

Cleaning the rotor and buckets

- 1. Switch off the QIAcube at the power switch.
- 2. Remove used disposable labware, sample tubes, and reagents from the worktable. Discard according to your local safety regulations.
- 3. Close the buffer bottles tightly, and store according to the instructions in the relevant kit handbook.
- 4. Remove all disposable rotor adapters, including tubes and spin columns, from the buckers
- 5. Remove the buckets from the rotor. Undo the rotor nut on top of the rotor using the rotor key, and carefully lift the rotor off the rotor shaft.
- 6. Submerge the rotor buckets, and rotor nut in cleaning agent. Incubate as appropriate.
- 7. Rinse thoroughly with distilled water. Use a brush (i.e., a toothbrush or tube brush) to clean any parts that are difficult to access such as the bucket mount and the rotor head. Wipe surfaces dry with a soft lint-free cloth. If available dry the buckets and rotor with pressurized air.





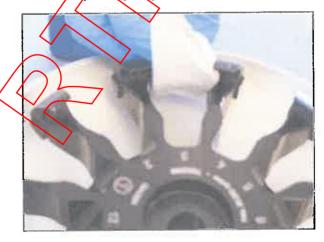
Brushing the rotor.

Important: Make sure the paper towels and brush used are lint-free.

Important: Make sure that all residual salt is removed. Important: Make sure to remove all traces of cleaning agent from the centrifuge buckets. Residual agent can cause the buckets to jam.

- 8. Apply a few drops of mineral oil (Anti-Corrosion Oil (rotor), cat. no. 9018543) on a soft, lint-free cloth, and wipe the bucket mount and rotor claw. A thin, invisible oil film should cover the bucket mount and rotor claw, but no droplets or smear should be apparent.
- 9. Apply oil to the rotor claw and to the bucket mount.

Important: Before applying oil to the rotor buckets on the rotor, make sure that the rotor and all buckets are completely dry.



Rotor head.



Bucket mounts.

Cleaning the centrifuge

- 1. Moisten a soft lint-free cloth with cleaning agent, and clean the inside of the centrifuge and the centrifuge gasket. Incubate as appropriate.
- 2. Clean the inside of the centrifuge and the gasket with distilled water, and wipe dry with lint-free paper towels. If available, use a vacuum cleaner.

 Important Make sure the gaskets remain in the proper positions.
- 3. Clean the centrifuge lid with a soft lint-free cloth moistened with cleaning agent. Incubate as appropriate, clean with water, and wipe dry with paper towels.
- A. Check the centrifuge gasket for damage. If the gasket is damaged or shows signs of wear, contact QIAGEN Technical Services.

Installing the centrifuge rotor and buckets

 $\overline{\mathbb{N}}$ Mount the rotor.

The rotor can be mounted in only one orientation. The pin on the rotor shaft fits into a notch on the underside of the rotor directly underneath rotor position 1. Line up position 1 of the rotor with the pin on the rotor shaft and carefully lower the rotor onto the shaft. Install the rotor nut on top of the rotor and tighten using the rotor key

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supplied with the QIAcube. Make sure that the rotor is securely seated.

WARNING



Risk of personal injury and material damage [W17]
The rotor nut must be securely tightened using the rotor key supplied with QIAcube. If the rotor nut is not tightened properly, it can become loose during operation of the centrifuge.

2. Replace the rotor buckets.

When replacing the rotor buckets, the side of the rotor bucket that must face toward the rotor shaft is marked with a gray line. Hold the bucket at an angle with the gray line facing the center of the rotor and hang the bucket on the rotor. Check that all buckets are properly suspended and can swing freely.

Important: All centrifuge buckets must be mounted before starting a run

6.4.2 Cleaning the instrument

To clean the instrument, perform the monthly maintenance procedure (Section §.3).

6.4.3 Tightness test

The tightness test is performed to check whether the tightness of the pipetting system, including the attached pipetting tip, is sufficient.

- 1. Load an empty 2 ml safe- lock microcentrifuge tube in position 1 of the shaker.
- 2.) Fill a reagent bottle with 96% ethanol and place in position 1 of the reagent bottle rack.
- 3. Load a tip rack of the filter tips you want to test (1000 μ l or 1000 μ l wide-bore) onto the QlAcube.
- 4. Make sure that the QIAcube is switched on.
- 5. In the main menu, press "Tools".

- Select "Maintenance" by pressing "▲" or "▼" to scroll through the list until it is highlighted, and then press "Select".
- 7. Select "Tightness test" by pressing "♠" or "♥" to scroll through the list until it is highlighted, and then press "Select".
- 8. Select the appropriate type of filter-tips ("1000 μl tips" or "1000 μl wide-bore tips") by pressing "♠" or "▼" to scroll through the list until it is highlighted, and then press "Select".
- 9. Press "Start" to start the tightness test with the selected type of filter-tips.
- 10. Follow the instructions displayed in the touchscreen, and press "Start" to start the tightness test.

 After the load check, the robotic arm will pick up a tip, aspirate ethanol, and move to the tube. The tip will remain in place above the tube for 2 minutes. The tip will be detached.
- 11. After the protocol is completed, open the QlAcube door and check if the tube contains liquid. If the tube is still empty and dry, the tightness of the pipetting system is adequate. If you find liquid in the tube, contact QlAGEN Technical Services.

6.5 Decontaminating the QIAcube

If the QlAcube is contaminated with infectious material, it should be decontaminated. The QlAcube should also be decontaminated before shipping. In this case, a decontamination certificate must be completed to confirm that the decontamination procedure has been carried out.

To decontaminate the QIAcube, follow the periodic maintenance procedure in Section 6.4, using the recommended disinfection agents.

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6.6 Cleaning the QIAcube centrifuge after a plastic crash

WARNING

Risk of personal injury

[W23]



After a plastic crash, sharp plastic particles could be inside the centrifuge. Be careful when handling items inside the centrifuge.

Select the appropriate cleaning agent according to the sample material and downstream assay (see Section 6).

6.6.1 Opening the centrifuge lid

The centrifuge lid must be open to allow access to the inside of the centrifuge. The lid should be opened only after the centrifuge has come to a complete stop. If the lid does not open automatically, perform the following steps.

- 1. Press "Tools" in the main menu.
- 2. Highlight "Maintenance" (sero) through the list by pressing "A" or " and press "Select".
- 3. Highlight "Open lid" (scroll through the list by pressing "A" or "V") and press "Select".

6.6.2 Cleaning the rotor and buckets

- 1. Switch off the QlAcube at the power switch.
- 2. Remove used disposable labware, sample tubes, and reagents from the worktable. Discard according to your local safety regulations.
- 3. Close the buffer bottles tightly, and store according to the instructions in the relevant kit handbook.
- 4. Remove all disposable rotor adapters, including tubes and spin columns, from the buckets.
- Remove the buckets from the rotor. Undo the rotor nut on top of the rotor using the rotor key, and carefully lift the rotor off the rotor shaft.
- 6. Submerge the rotor, buckets, rotor nut, and rotor key with a cleaning agent. Incubate as appropriate.

7. Rinse thoroughly with distilled water. Use a brush (i.e., a toothbrush or tube brush) to clean any parts that are difficult to access, such as the bucket mount and the rotor head (see figures in Section 6.4.1). Wipe surfaces dry with a soft lint-free cloth. If available, dry the buckets and rotor with pressurized air.

We recommend drying using pressurized air to remove any small plastic particles adhering to the rotor or the buckets. When handling the bucket, pay particular attention that the bucket mount is not damaged.

Important: Be sure to only use lint-free paper towels and brushes.

Important: Make sure all residual plastic and salt are removed.

Important: Make sure all traces of cleaning agent are removed from the centrifuge buckets. Residual disinfectant can cause the buckets to jam.

8. Apply a few drops of mineral oil (Anti-Corrosion Oil (rotor), cat. no. 2018543) on a soft, lint-free cloth, and wipe the bucket mount and rotor claw. A thin, invisible oil film should cover the bucket mount and rotor claw, but no droplets or smear should be apparent.

Important: Before applying oil to the rotor buckets on the rotor, make sure that the rotor and all buckets are completely dry.

6.6.3

Cleaning the centrifuge

Moisten a soft lint-free cloth with cleaning agent, and clean the inside of the centrifuge and the centrifuge gasket. Incubate as appropriate.

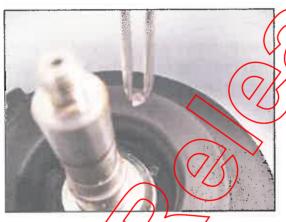
Note: Do not spray disinfectant into the centrifuge.

Clean with water, and wipe dry with lint-free paper towels. If available, use a vacuum cleaner to remove any small plastic particles from the inside of the centrifuge. Tweezers or tape can be used to remove plastic particles from any difficult to access parts. Special care must be taken to remove any plastic particles from the rotor pegs, floor and back of the centrifuge compartment, and the rotor shaft guide.

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Removing plastic debris with tape.

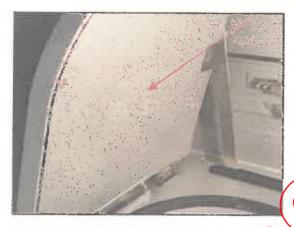


Removing plastic debris with tweezers.

Important: Make sure to use lint-free paper towels. Important: Make sure that all plastic particles are removed.

important: Make sure the gaskets remain in the proper positions.

Clean the centrifuge lid with a soft lint-free cloth moistened with a cleaning agent. Incubate as appropriate, and wipe dry with paper towels.



Centrifuge lid with plastic dust.

- 4. Check if plastic particles are below the gasket.
- 5. Check the centrifuge gasket for damage. If the gasket is damaged or shows signs of wear, contact QIAGEN Technical Services.

6.6.4 Cleaning the workingle

- 1. Empty the waste drawer and check that the liner is clean. If necessary clean with cleaning agent, and rinse with distilled water.
- 2. Clean the shaker rack, labware tray, heating adapter, and reagent bottle rack with cleaning agent. Rinse thoroughly with distilled water, and wipe dry with paper towels.
 - Clean the surface of the worktable using a soft lint-free cloth moistened with cleaning agent. Incubate as appropriate, clean with water and wipe dry with lint-free paper towels.
 - Do not spray liquid onto the worktable. Take particular care not to spray liquid onto the display.
 - Important: Make sure to use lint-free paper towels.
 Important: Make sure that every plastic item is wiped off.





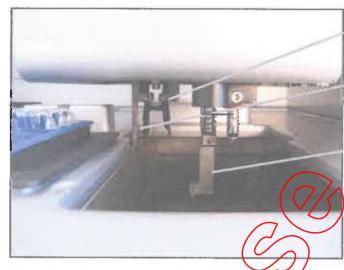
Centrifuge and worktable after deaning.

6.6.5 Cleaning the gripper unit

- 1. Switch on the QIAcube on the power switch
- 2. Moisten a soft lint-free cloth with 70% ethanol.
- 3. Press "Tooks" in the main menu.
- 4. Highlight "Maintenance" (scroll through the list by pressing "A" or "V"), and press "Select".
- 5. Highlight "Cleaning position" (scroll through the list by pressing "∧" or "∀"), and press "Start".
- 6. Follow the instructions displayed in the touchscreen.
 You will be asked to remove the waste drawer and the labware tray.
 - The robotic arm will move forward and down, enabling the gripper unit to be accessed for cleaning through the opening for the waste drawer.
- 7. Carefully wipe the gripper unit, including the gripper, the stabilizing rod, and the spin column lid holder.

 Important: Make sure to use lint-free paper towels.

 Ensure that any residual salt is removed.



Robotic gripper

Spin column lid holder

Stabilizing rod

Cleaning the gripper unit.

6.6.6 Installing the centrifuge rotor and buckets

1. Mount the rotor.

The rotor can be mounted in only one orientation. The pin on the rotor shaft fits into a notch on the underside of the rotor directly underneath rotor position 1. Line up position 1 of the rotor with the pin on the rotor shaft and carefully lower the rotor onto the shaft. Install the rotor nut on top of the rotor and tighten using the rotor key supplied with the QIAcube. Make sure that the rotor is securely seated.

WARNING

Risk of personal injury and material damage [W17]
The rotor nut must be securely tightened using the rotor key supplied with QIAcube. If the rotor nut is not tightened properly, it can become loose during operation of the centrifuge.

Replace the rotor buckets.

When replacing the rotor buckets, the side of the rotor bucket that must face toward the rotor shaft is marked with a gray line. Hold the bucket at an angle with the gray line facing the center of the rotor and hang the bucket on the rotor. Check that all buckets are properly suspended and can swing freely.

Note: The side of the rotor bucket that must face towards the rotor shaft is marked with a gray line to help prevent buckets from being loaded incorrectly.

Important: All centrifuge buckets must be mounted before starting the test run.

6.6.7 Operating the centrifuge after cleaning

The centrifuge must be operated independently before starting further protocol runs to test if residual plastic parts are still in the centrifuge.

For detailed information about operating the centrifuge, see Section 5.8.

- 1. Switch on the QlAcube at the power switch.
- 2. Set centrifugation parameters for a centrifuge test protocol.

Press "Tools" in the main meny.

Highlight "Centrifuge" (scroll through the list by pressing "A" or "∀", and press "Select".

To select the speed, time, and acceleration, press "Edit" and set the following parameters.

- Time 1/1: 60 sec
- "Speed 1":/12,000 g
- "Acceleration": 9
- "Time 2": 0 sec
- Speed 2": default

Press "Back" to exit edit mode.

Press "Start" to start the centrifuge.

Note: Rotor adapters and other consumables are not required.

Carefully listen to the sound during centrifugation.

Unusual sound during centrifugation:

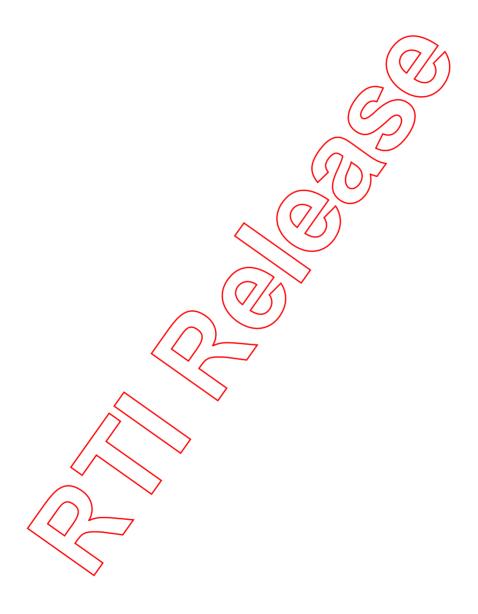
Yeany grinding, rattling, or crunching sounds are heard during the centrifugation, there are still loose plastic particles inside the centrifuge. Repeat the cleaning procedure described in Section 6.6.

Note: it may be necessary to repeat the procedure several times to remove all plastic particles.

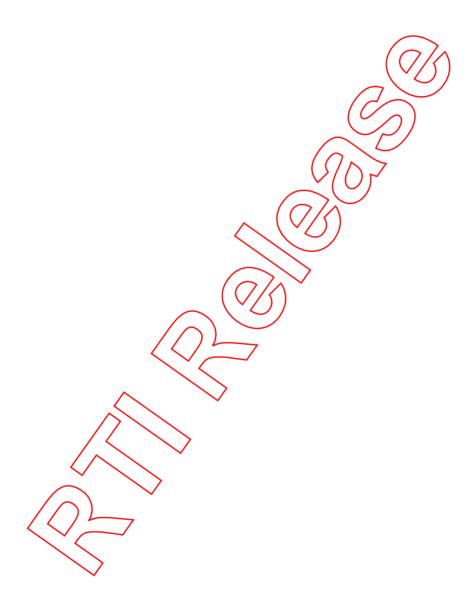
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No unusual sound during centrifugation:

If no unusual sound from loose plastic particles can be heard during centrifugation, the next sample run can be started.



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7 Troubleshooting

7.1 General information

Whenever encountering a QIAcube error, be sure to have the following information at hand:

- Protocol name and version (found in the report file)
- Software version (see Section 5.5)
- Sample input material
- Detailed description of the error situation

This information will help you and your QIAGEN Technical Service Specialist to deal most efficiently with your problem.

Note: Information about the latest software and protocol versions can be found at www.qiagen.com/QIAcube/Resources. In some cases, updates may be available for addressing specific problems.

7.2 Resolving error messages and warnings

Error messages or warnings are displayed in the touchscreen if a problem occurs during operation. Refer to the error codes listed in this section if an error message or warning appears. If error messages or warnings appear that are not listed here or if the error cannot be resolved, note down the error code and description and the steps leading to the error, and contact QIAGEN Technical Services.

Note: It is only possible to continue a protocol run that has been interrupted due to an error if the error occurs before the protocol has started (e.g., during the load check). If an error occurs during sample processing, the samples must be processed manually (see Section 7.2.6).

If an error occurs during the load check, the protocol run can be continued as soon as the error has been resolved.

Note: When resolving a load check error, only move or change the affected samples or consumables.

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Load check errors 7.2.1

Error code	Description	Comments and suggestions
301	Optical sensor error: invalid teaching	Check worktable for loose plasticware. Clean the optical sensor.
302	Optical sensor error: invalid measurement	Clean the optical sensor.
317, 320	Ultrasonic sensor error	Make sure that there is no foam in the reagent bottles. Check that the bottles contain sufficient reagent and are in the correct positions in the reagent bottle rack. Make sure that the labeling strips are attached correctly (Section 5.3.1).
3200	Load check error: invalid rotor loading	Check that the rotor is symmetrically loaded. Clean the optical sensor.
3201	Load check error: not enough buffer	Check the volume of reagent in the reagent bottles. Make sure that the bottles are in the correct positions in the reagent bottle rack.
3202	Load check error: buffer empty, too much buffer, or capped buffer bottle	Check the volume of reagent in the reagent bottles. The maximum volume is 30 ml and is indicated by a calibration mark. Make sure that the caps have been removed from the reagent bottles.
3203	Load check error: too few wide-bore 1000 μ l tips	Make sure to load sufficient $1000 \mu l$ wide-bore filter-tips for the protocol run.
3204	Load check error: too few $1000~\mu$ l tips	Make sure to load sufficient 1000 μ l filter-tips for the protocol run.

Error code	Description	Comments and suggestions
3205	Load check error: too few $200 \mu l$ tips	Make sure to load sufficient 200 μ l filter-tips for the protocol run.
3206	Load check error: invalid shaker loading	Check that the shaker is correctly loaded (see Section 5.3.4) and the sample tubes are in the correct position (see Appendix B). In particular, make sure that the latest version of the shaker rack plug is being used and that only loaded positions are marked. Clean the optical sensor.
3207	Load check error: too much buffer in bottle	Check that the reagent bottle is not overfilled. The maximum volume is 30 ml and is indicated by a calibration mark.
3208	Load check error: no sample tubes or invalid sample tubes detected	Check that samples have been loaded onto the shaker. Clean the optical sensor. Check the worktable for obstructions that might prevent movement of the shaker. Make sure that the correct shaker adapter is installed.
3212	Load check errors remove labware tray	Remove the labware tray and start the "Cleaning position" protocol again.
3302	Error: instrument door is	Make sure that the QIAcube door is properly closed.
		Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
3303	Error: picked up wrong tip	Check that tip racks are correctly loaded (see Section 5.3.2).

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Error code	Description	Comments and suggestions
3304	Error: picked up no tip	Clean the tip sensor. When using a plain white 200 μ l tip rack, always fill the tip rack with 200 μ l tips. Alternatively, replace the white rack with the optimized blue rack for 200 μ l tips.
7.2.2	Protocol errors	
Error code	Description	Comments and suggestions
403	Protocol error: protocol not found	Reload the protocol from the USB stick (see Section 5.4) Switch off the Olecube, wait for a few minutes, and switch it back on again. If
		the error persists, contact QIAGEN Technical Services.
404	Protocol error: invalid protocol data format (checksum error)	Delete the protocol from the QIAcube. Download the protocol from the QIAGEN website
	(CHECKSOITI EITOI)	to the USB stick. Reload the protocol to the QIAcube (see Section 5.4).
		Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
	\sim	

Error code	Description	Comments and suggestions
405	Protocol error: unable to save protocol	Delete protocols from the QlAcube that are no longer required and reload the protocols from the USB stick (see Section 5.4).
		Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
406	Protocol error: maximum number of protocols	Delete protocols from the QIAcube that are no longer/required (see Section 5.4). Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
407	Protocol error: unable to save protocol list	Delete protocols from the QIAcube that are no longer required (see Section 5.4).
		Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
408	Protocol error: max. protocol steps exceeded	Contact QIAGEN Technical Services for a new protocol.
409	Protocol error: missing liquid definition	Contact QIAGEN Technical Services for a new protocol.
410	Protocol error: missing vessel definition	Contact QIAGEN Technical Services for a new protocol.
412	Protocol error: missing working space definition	Contact QIAGEN Technical Services for a new protocol.

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Error code	Description	Comments and suggestions
414	Protocol error: missing liquid class definition	Contact QIAGEN Technical Services for a new protocol.
415	Protocol error: missing liquid definition	Contact QIAGEN Technical Services for a new protocol.
416	Protocol error: invalid protocol parameter	Contact QIAGEN Technical Services for a new protocol.
417	Protocol error: parameter type mismatch	Contact QIAGEN Technical Services for a new protocol.
418	Protocol error: invalid protocol version	Load the protocol again. If the error persists, contact QIAGEN Technical Services.
419	Protocol error: invalid protocol version	Protocol required new QIAcube firmware. Delete all protocols before updating. Load the protocol again. If the error persists, contact QIAGEN Technical Services. The most recent software versions can be downloaded from www.qiagen.com/QIAcube/Resources .
1000 <u></u> 1022	Protocol error	Contact QIAGEN Technical Services for new protocols.

7.2.3 File system errors

Error code	Description	Comments and suggestions
700	File system error: unable to delete data	Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
701	File system error: copy error to USB	Switch off the Qlacube wait for a few minutes, and switch it on again. Save the file(s) to the USB stick again. If the error persists, contact QIAGEN Technical Services.
702	File system error: missing USB device	Switch off the QIAcube, wait for a few minutes, and switch it on again. Insert the USB stick into the USB port. If the error persists, contact QIAGEN Technical Services.
703	File system error: unable to read data	Switch off the QIAcube, wait for a few minutes, and switch it on again. Save the file(s) to the USB stick again. If the error persists, contact QIAGEN Technical Services.
704	File system error: flashdisk is full	Delete protocols from the QIAcube that are no longer required. If the error persists, contact QIAGEN Technical Services.
705/	File system error: checkdisk error	Memory error or memory defective indicating that the USB stick may be partially or entirely corrupted. Format the USB stick according to instructions at www.qiagen.com/QIAcube/Resources .

7.2.4 **General instrument errors**

Error code	Description	Comments and suggestions
1–33	Internal motion error	Identify and make a note of the axis in error (as listed in the error message). Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
50-52	Motor driver error: overload/no motor detected/power surge	Identify and make a note of the axis in error (as listed in the error message). Switch off the QIAcube and check the worktable for damage or evidence of a collision. If the error persists, contact QIAGEN Technical Services.
53	Motor driver error: overheat prewarning	Make sure that the QIAcube is operating under the correct conditions (see page 1-3 and Appendix A).
54	Motor driver error: overheat	Make sure that the QIAcube is operating under the correct conditions (see Section 4.1 and Appendix A).
70	Motion error: stopped in limit switch	Identify and make a note of the axis in error (as listed in the error message). Switch off the QIAcube and check the worktable for loose plasticware.
80	Homing error: reference switch failure on <> axis	Identify and make a note of the axis in error (as listed in the error message). Switch off the QlAcube and check the worktable for loose plasticware. If the error persists, contact QlAGEN Technical Services.

Error code	Description	Commonto and averagetions
303	Memory module: RAM test failure	Switch off the QlAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QlAGEN Technical Services.
330– 331	Internal shaker/heater error	Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
340– 359	Internal centrifuge error	Contact QIAGEN Technical Services.
500- 502	Program not ready or in error state	A protocol cannot be started if the previous instruction is not completed and the instrument is busy.
		Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
600	Logging error: buffer overflow	Although this code is presented as an error, it is a warning and can be ignored. It will not result in protocol interruption.
900- 999	Firmware update error	Please contact QIAGEN Technical Services.
2000	General error: PLC programming error	Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.

Error code	Description	Comments and suggestions
3000	Centrifuge error: failed positioning	Make sure that the rotor is installed. Check that the rotor is correctly positioned on the rotor shaft.
		Switch off the QlAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QlAGEN Technical Services.
3001	Centrifuge error: invalid g range	Contact QIAGEN Technical Services for a new protocol.
3101	Motion error: invalid rotation axis move	Switch off the OlAcube, wait for a few minutes, and switch it back on again. If the error persists, contact OlAGEN Technical Services.
3102	Motion error: invalid disposal position	Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIACEN Technical Services.
3103	Motion error: invalid Zaxis	Check the worktable for obstructions.
	move	Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
3104	Motion error: invalid tip	Make sure that the tip rack is not damaged and is correctly positioned on the worktable.
		Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.

Error code	Description	Comments and suggestions
3106	Motion error: tip detection failed	Check to see if there are loose tips on the worktable or if the rack still contains tips that should have been picked up.
		Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
3108	Motion error: invalid gripper position	Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
3109	Motion error: homing limit reached, no light barrier found	dentify and make a note of the axis in error (as listed in the error message). Check the worktable for obstructions.
		Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
3110	Motion error: no motion profile found for XY move	Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.

Error		
code	Description	Comments and suggestions
4000	Motion error: step loss on one axis	Identify and make a note of the axis in error (as listed in the error message). Check the worktable and the centrifuge for obstructions. Make sure that the correct plasticware was used. Check that all plasticware is loaded in the correct positions and is seated firmly.
		Switch off the QlAcube and carefully pull the robotic arm forward. Check that the tip disposal slot is not blocked. Make sure that a disposable tip is not stuck to the tip adapter or that a spin-column or microcentrifuge tube is not stuck to the robotic gripper. If an item is stuck, reinitialize the QlAcube or contact QlAGEN Technical Services. If the problem persists, contact QlAGEN Technical Services.
5024– 5027	Centrifuge error: lid drive error	Make sure that nothing is obstructing the centrifuge lid.
		Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.
5031	Centrifuge error motor overheat	Make sure that the QIAcube is operating under the correct conditions (see Section 4.1 and Appendix A).
		Switch off the QIAcube, and wait 10 min before switching it back on. If the problem persists, contact QIAGEN Technical Services.

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Error		
code	Description	Comments and suggestions
5032	Centrifuge error: electronics overheat	Make sure that the QIAcube is operating under the correct conditions (see Section 4.1 and Appendix A).
		Switch off the QIAcube, and wait 10 min before switching it back on. If the problem persists, contact QIAGEN Technical Services.
5129	Centrifuge error: imbalance detected	Make sure that the rotor is symmetrically loaded. Remove the rotor and check the centrifuge chamber for loose plasticware.
		Switch off the QIAcube, wait for a few minutes, and switch it back on again. If the error persists, contact QIAGEN Technical Services.

7.2.5 General troubleshooting

Comments and suggestions

Rotor adapter deformed or lid of spin-column broken off

Disposable rotor adapter not properly loaded

Remove the rotor and buckets from the centrifuge. Remove plastic shards from the centrifuge. Clean the rotor and buckets and replace in the centrifuge as described in Section 6.0

Errors in pipetting or drops of buffer on the worktable

Worktable loaded incorrectly

Check that the reagent bottles contain the correct buffers and are correctly placed in the reagent bottle rack. Be sure to use the right plasticware. Check the volumes in the sample tubes and tubes of accessory buffer(s), if applicable. Do not exceed the recommended amount of starting material to availablocking disposable filter-tips. If tip racks have been refilled, make sure that the correct tips were used.

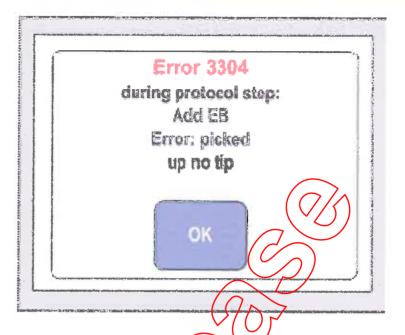
System tightness issue

Perform system tightness test (see Section 6.4.3).

7.2.6 Protocol interruption

If an error occurs during a protocol run, it is possible to continue sample preparation manually. The error code, the description, and the step at which the protocol stopped are displayed in the touchscreen. In the example below, the protocol stopped during addition of Buffer EB to the samples.





To continue sample processing:

- 1. Note the step at which the protocol stopped. This is displayed in the touchscreen.
- 2. Remove the samples and reagents from the QIAcube.
- 3. Refer to the appropriate protocol in the relevant kit handbook and continue sample processing manually.

7.2.7 Centrituge

Opening the centrifuge lid in the event of a breakdown

In case of power failure, the centrifuge lid can be manually opened so that the samples can be removed. To open the centrifuge lid, follow the instructions below.



Moving parts

In case of breakdown caused by power failure, remove the power cord and wait 10 minutes before attempting to manually open the centrifuge lid.

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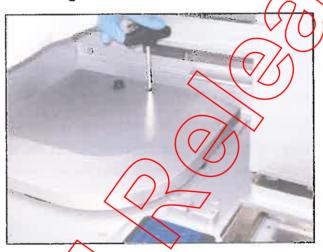
W121

WARNING



Risk of personal injury and material damage [W13] Raise the centrifuge lid carefully. The lid is heavy and may cause injury if it falls down.

- 1. Switch off the QIAcube.
- 2. Unplug the power cord from the power outlet. Wait 10 min for the rotor to stop.
- 3. Open the instrument door.
- 4. Carefully move the robotic arm to the right side of the worktable, furthest away from the centrifuge lid
- 5. Remove the screw protection on top of the centrifuge lid.
- 6. Using the rotor wrench, turn the screw counter clockwise.



Turning the screw in the centrifuge lid.

7. Remove the waste drawer.

A white disk that is attached to the end of the centrifugelid release cord will be visible through the waste drawer
compartment. The disk can be accessed by reaching
through the opening for the waste drawer.



Accessing the centrifuge-lid release cord,

8. Grasp the disk and pull to access to the cord. Pull the cord firmly to release the higher the lock.



Rulling the centrifuge-lid release cord.

- 9. Manually raise the centrifuge lid.
- 10. Hold the raised lid and remove the samples from the rotor.



Removing samples.

11. Contact QIAGEN Technical Services for instructions on how to reset the lid.

increased sample temperature

Check the slits and openings at the bottom and the rear of the instrument.

WARNING



[W14]



To ensure proper ventilation, maintain a minimum clearance of 10 cm at the sides and rear of the QIAcube. Slits and openings that ensure the ventilation of the QIAcube must not be covered.

- Slits and openings for ventilation must not be covered.
- You should be able to detect an air-flow at the openings.

 If an air-flow cannot be detected, contact QIAGEN

 Technical Services.

Excessive noise

Excessive noise during centrifugation may indicate that there are loose parts inside the centrifuge or that the rotor is not balanced.

- Press "Cancel" to stop the protocol run.
- 2. When the centrifuge has come to a complete stop, the lid should open. If the lid does not open:

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- Press "Tools" in the main menu.
- Select "Maintenance" by pressing "A" or "▼" to scroll through the list until it is highlighted, and then press "Select".
- Select "Open lid" by pressing "▲" or "▼" to scroll through the list until it is highlighted, and then press "Select".
- 3. Remove the buckets from the rotor Undo the rotor nut on top of the rotor using the rotor key and carefully lift the rotor off the rotor shaft.
- 4. Check that there are no loose parts inside the centrifuge or on the rotor. Check that the rotor is loaded symmetrically. Check that the rotor, buckets, and gasket are not damaged.
- 5. If there are loose parts inside the centrifuge or on the rotor, clean according to Section 6.6.

Liquid spills in the centrifuge

The rotor adapter is designed for use with QIAGEN automated protocols. Do not fill the rotor adapters with liquid.

Liquid spills may occur if QIAGEN spin columns become blocked due to sample overloading. Do not exceed the maximum amount of starting material.

rotor adapters to leak. Check that the buckets are installed properly and can swing freely (see page 4-6).

If there is a liquid spill in the centrifuge, clean according to the instructions in Section 6.4.1.



7.2.8 Shaker

Sample temperature too low

To ensure efficient heat transfer, make sure to use the correct shaker adapter. Choose the appropriate shaker adapter for the sample tubes:

- Adapter for 2 ml microcentrifuge tubes (2)
- Adapter for 2 ml screw-cap tubes (S2)

7.2.9 Touchscreen

Display

The QIAcube beeper should sound and the startup screen should be displayed when the QIAcube is switched on. If this does not happen, check that the power cord is properly connected.

If the QIAcube beeper sounds when the QIAcube is switched on but the startup screen is not displayed, contact QIAGEN Technical Services.

If the display does not react when pressed, improper cleaning procedures might have caused liquid to enter the space between the display sheets and liquid might be visible in sections of the touchscreen. Contact QIAGEN Technical Services.

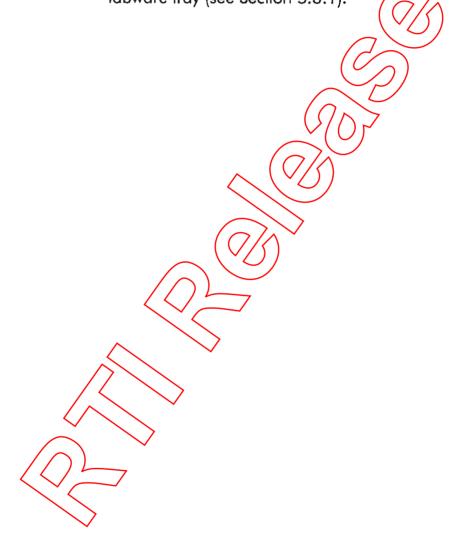
7.2.10 USB stick

Only use USB sticks supplied by QIAGEN. Other USB sticks may not work with the QIAcube.

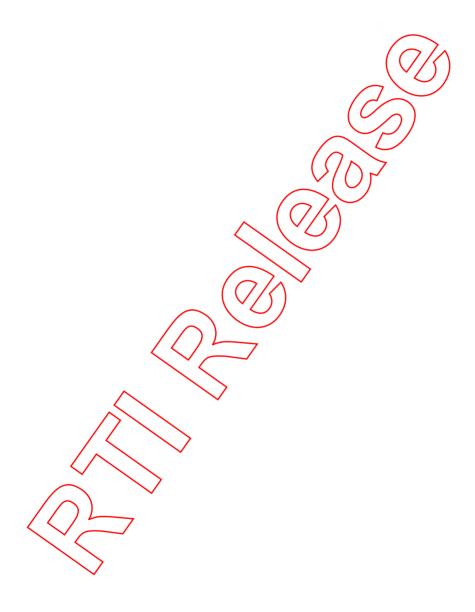
7.2.11 Reagent volume detection

To help prevent errors during detection of reagent volumes, make sure that both rack labeling strips are attached to the reagent bottle rack. These strips ensure that the reagent bottle rack is positioned correctly on the worktable for liquid detection during the load check.

Also make sure that the bottle rack is sitting correctly in the labware tray (see Section 5.3.1).



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Term	Description
Centrifuge	A component of the QlAcube that accommodates a rotor with 12 swing-out buckets. Each bucket holds a disposable rotor adapter.
Column disposal slot	A slot in the QIAcube worktable through which used columns (e.g., QIAshredder columns) are discarded into the waste drawer.
Dilutor unit	A module consisting of a syringe pump (syringe, dilutor valve, motor) and a tip adapter. The dilutor unit is for high-precision aspiration and dispensing of liquid.
Disposal slots	Slots in the QIAcube worktable through which used tips and columns (e.g., QIAshredger columns) are discarded into the waste drawer.
Door	The main door at the front of the QIAcube. When open, it provides complete access to the worktable.
Error code	A 3 or A digit number that indicates a particular error of the QIAcube.
Filter-tip	An item of labware that is picked up by the tip adapter during operation of the QIAcube. Liquid is aspirated into and dispensed from a filter-tip.
Initialization	An operation performed automatically when the QIAcube is switched on and before each protocol run to check the operation of the QIAcube.
Labware tray	An item that accommodates the reagent bottle rack, the tip racks, and has 3 slots for accessory buffers in 1.5 ml or 2 ml microcentrifuge tubes.

Microcentrifuge Three slots located in the labware tray that accommodate tube slots accessory buffers, in 1.5 ml or 2 ml microcentrifuge tubes.

Relative humidity Max. 75% (noncondensing)

Environmental 2K2 & 2M2 (IEC 60721-3-2)

class

Storage conditions

Air temperature 15°C to 30°C (59°F to 86°F) in manufacturer's pockage

Relative humidity Max. 75% (noncondensing)

Environmental 1K2 (IEC 60721-3-1)

class

Mechanical data and hardware features

Dimensions Width: 65 cm (25.6 in.)

(door closed) Height: 57 cm (22.4 in.)

Depth: 62 cm (24.4 in.

Dimensions Width: 65 cm (25.6 in.) (door open) Height: 81 cm (32 in.)

Depth: 62 cm (24.4) in)

Mass QIAcube: 71.5 kg (157.6 lb.)

Accessories: 3 kg (6.6 lb.)

Centrifuge 12,000 x g maximum

wing-out rotor, maximum 45°

12 rotor positions

Shaker Speed 100–2000 rpm

Heating range of ambient temperature to 70°C (158°F)

Ramp-up time of <5 minutes from ambient temperature to 55°C (±3°C)

Difference in the temperature detected by the internal sensor and the temperature of the sample liquid is approximately –2°C Pipetting

Syringe size 1 ml

system

Pipetting range 5–900 μl

Capacity

Up to 12 samples per run

Touchscreen

Transmissive TFT, $64 \times 86 \times 6.5$ mm, white LED backlight,

high brightness

Software

QIAGEN protocols are preinstalled on the QIAcube or can be downloaded at www.giagen.com/MyQtAcube.

Waste Electrical and Electronic Equipment (WEEE)

This section provides information about disposal of waste electrical and electronic equipment by users in the European Union.

The European Directive 2002/96/EC on WEEE requires proper disposal of electrical and electronic equipment when it reaches its end of life. The crossed-out wheeled bin symbol (see below) indicates that this product must not be disposed of with other waste; it must be taken to an approved treatment facility or to a designated collection point for recycling, according to local legislation. The separate collection and recycling of waste electronic equipment at the time of disposal helps to conserve natural resources and ensures that the product is recycled in a manner that protects human health and the environment.



QIAGEN accepts its responsibility in accordance with the specific WEEE recycling requirements and, where a replacement product is being supplied by QIAGEN, provides free recycling of its WEEE-marked electronic equipment in Europe. If a replacement product is not being purchased from QIAGEN, recycling can be provided upon request at additional cost. To recycle electronic equipment, contact your local QIAGEN sales office for the required return form. Once the form is submitted, you will be contacted by QIAGEN either to request follow-up information for scheduling collection of the electronic waste or to provide you with an individual quote.

FCC declaration

The "United States Federal Communications Commission" (USFCC) (in 47 CRF 15. 105) declared that the users of this product must be informed of the following facts and circumstances.

"This device complies with part 15 of the FCC:

Operation is subject to the tollowing two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation."

"This Class B digital apparatus complies with Canadian ICES-0003."

The following statement applies to the products covered in this manual, unless otherwise specified herein. The statement for other products will appear in the accompanying documentation.

NOTE: This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules and meets all requirements of the Canadian Interference-Causing Equipment Standard ICES-003 for digital apparatus. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and

used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that the interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into a outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

QIAGEN GmbH Germany is not responsible for any radio television interference caused by unauthorized modifications of this equipment or the substitution or attachment of connection cables and equipment other than those specified by QIAGEN Gmbt, Germany. The correction of interference caused by such unguithorized modification, substitution or attachment will be the responsibility of the user.

EC declaration of conformity

Name and address of the company

QIAGEN GmbH

QIAGEN Strasse 1

40724 Hilden

Germany

We herewith declare under our sole responsibility that the product

QIAcube, cat. nos. 9001292, -93, -94, -95

meets all applicable requirements of the following European Directives

Low Voltage Directive (LVD) 2006/95/EC

Electromagnetic Compatibility 2004/108/EC Directive (EMC)

Hombrechtikon, 7 May 2007

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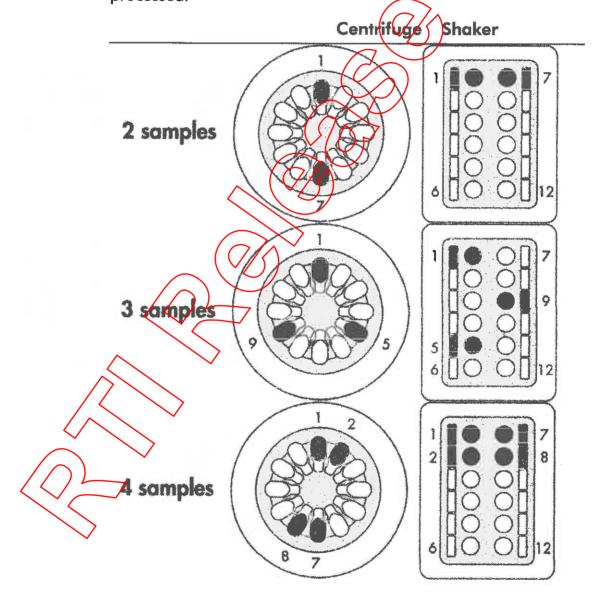
Director Business Excellence

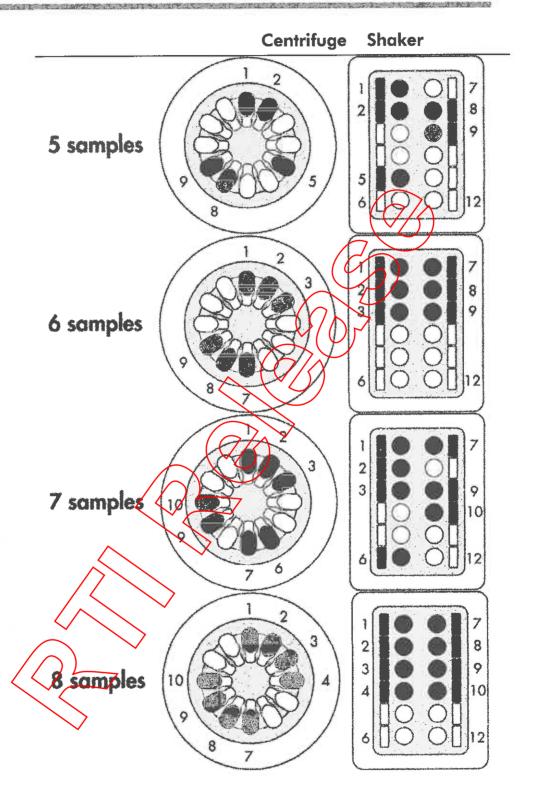
QIAGEN Instruments AG

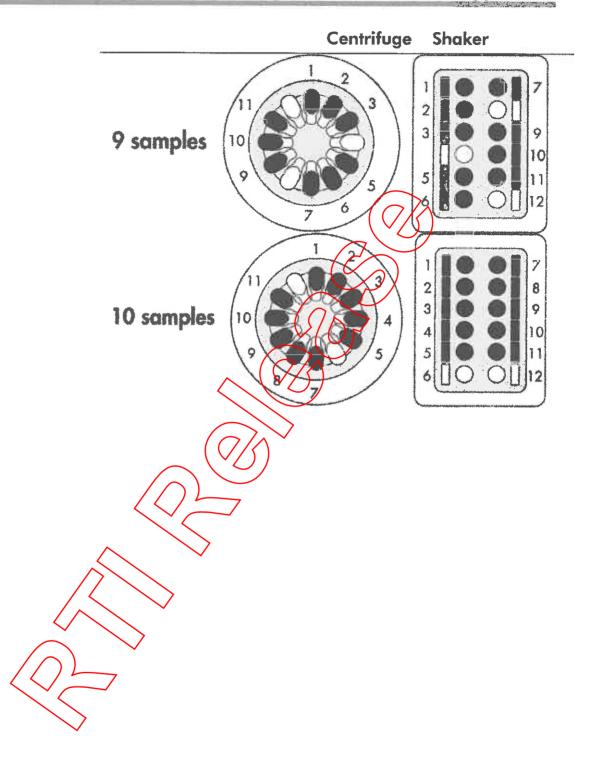
Appendix B

Loading the centrifuge and shaker

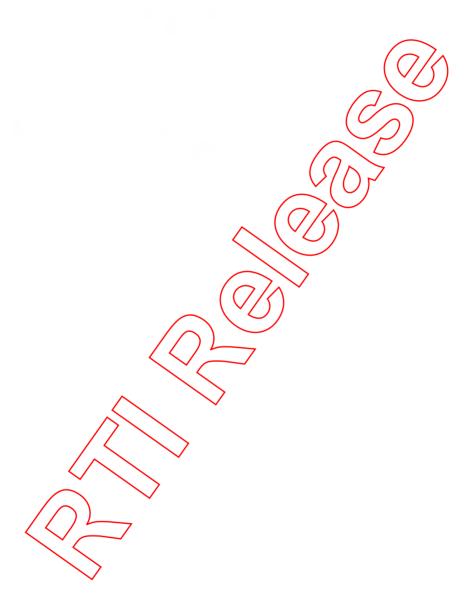
If fewer than 12 samples are to be processed, make sure to load the centrifuge and shaker correctly (see pictures below and following pages). One or 11 samples cannot be processed.







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Appendix C

QIAcube accessories

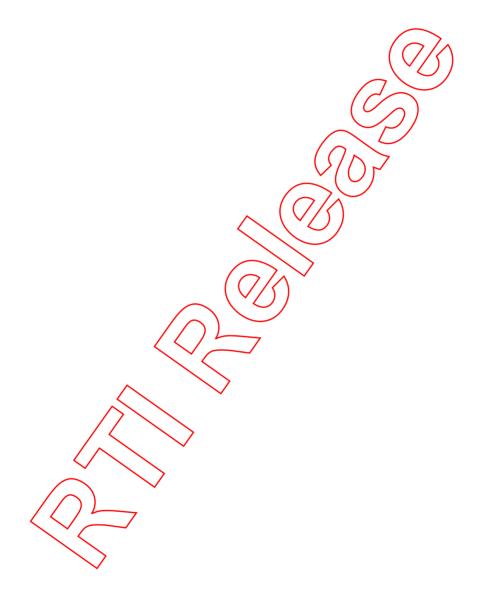
For more information and an up-to-date list of available protocols, visit www.qiagen.com/MyQlAcube.

Product	Contents	Cat. no.
QIAcube (110 V)*	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-	9001292* 9001293†
QIAcube (230 V) [†]	column kits, 1-year warranty on parts and labor [‡]	
Accessories	$\sqrt{2}\sqrt{2}$	
Starter Pack, QIAcube	Pack includes: reagent bottle racks (3); rack labeling strips (8); 200 µ/ tilten-tips (1024); 1000 µ/ filter-tips, (1024); 1000 µ/ filter-tips, wide-bore (1024); 30 m/ reagent bottles (18); rotor adapters (240) rotor adapter holder; 1.5 m/ microcentrifuge tubes (240)	990395
Filter-Tips, 1000 μl (1024)	Disposable Filter-Tips, racked; (8 x 128)	990352
Filter-Tips, $1000 \mu l$, wide-bore (1024)	Disposable Filter-Tips, wide-bore, racked; (8 x 128); not required for all protocols.	990452
Filter Tips, 200 µ (1024)	Disposable Filter-Tips, racked; (8 x 128)); not required for all protocols	990332

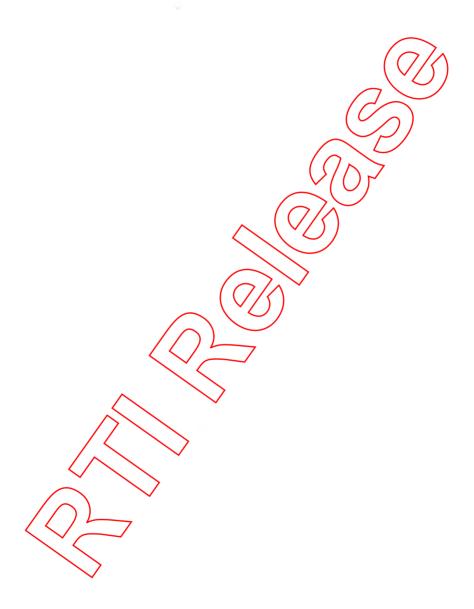
^{*} US, Canada, and Japan. † Rest of world. ‡ Agreements for comprehensive service coverage are available; please inquire.

Product	Contents	Cat. no.
Spin Column Adapter Rings (25)	Spin column adapter rings for use with spin- columns that have screw-caps; not required for all protocols	990399
Rotor Adapters (10 x 24)	For 240 preps: 240 Disposable Rotor Adapters and 240 microcentrifuge tubes (1.5 ml); for use with the QIAcube	990394
Rotor Adapter Holder	Holder for 12 disposable rotor adapters; for use with the QIAcube	990392
Reagent Bottle Rack	Rack for accommodating 6 x 30 ml reagent bottles on the QIAcube worktable	990390
Rack Labeling Strips (8)	Identification strips for the QIAcube reagent bottle rack; for use with RNeasy® Mini Kits, QIAamp® DNA Mini Kits, QIAquick® Kits, QIAprep® Spin Miniprep Kit, QIAamp MinElute® Virus Spin Kit, and Ni-NTA Spin Kit	990391
Rack Labeling Strips, Accessory Set	Additional identification strips for the QlAcube reagent bottle rack; for use with the miRNeasy Mini Kit, DNeasy® Blood & Tissue Kit, MinElute Reaction Cleanup Kit, MinElute PCR Purification Kit, MinElute Gel Extraction Kit, EpiTect™ Bisulfite Kit, and QlAcmp Viral RNA Mini Kit	990380
Reagent Bottles, 30 ml (6)	Reagent Bottles (30 ml) with lids; pack of 6; for use with the QIAcube reagent bottle rack	990393
Shaker Rack Plugs (12)	For use with 2.0 ml screw cap tubes	9017854
Sample Tubes RB (2 ml)	1000 safe-lock microcentrifuge tubes (2 ml) for use with the QIAcube	990381

Product	Contents	Cet. no.
Sample Tubes CB (2 ml)	1000 conical screw-cap tubes without skirted base (2 ml) for use with the QIAcube	990382
USB Stick	USB Stick; for use with the QIAcube	9017850



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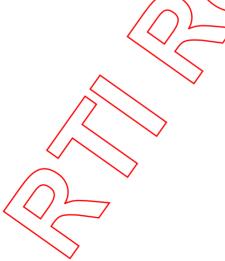


Appendix D

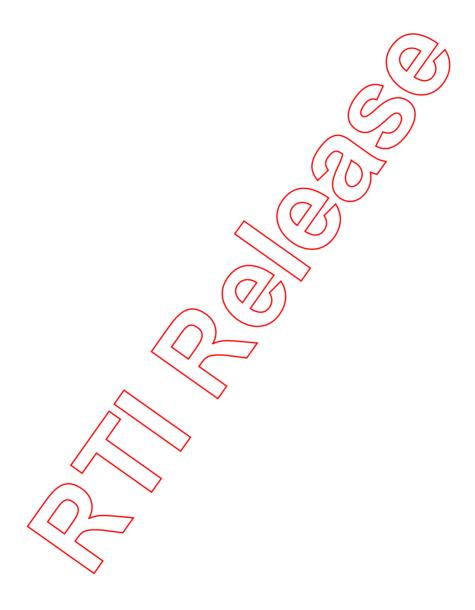
Liability clause

QIAGEN shall be released from all obligations under its warranty in the event repairs or modifications are made by persons other than its own personnel, except in cases where the Company has given its written consent to perform such repairs or modifications.

All materials replaced under this warranty will be warranted only for the duration of the original warranty period, and in no case beyond the original expiration date of original warranty unless authorized in writing by an officer of the Company. Read-out devices interfacing devices and associated software will be warranted only for the period offered by the original manufacturer of these products. Representations and warranties made by any person, including representatives of QIAGEN, which are inconsistent or in conflict with the conditions in this warranty shall not be binding upon the Company unless produced in writing and approved by an officer of QIAGEN.



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Australia Orders 03-9840-9800 Fax 03-9840-9888 Technical 1-800-243-066

Austria Orders 0800/28-10-10 Fax 0800/28-10-19 Technical 0800/28-10-11

Belgium Orders 0800-79612 Fax 0800-79611 Technical 0800-79556

Canada Orders 800-572-9613 Fax 800-713-5951 Technical 800-DNA-PREP (800-362-7737

China Orders 0086 21 3865 3865 Fax 0086 21 3865 3965 Technical 800 988 0325, 800 988 0327

Denmark * Orders 80-885945 Fax 80-885944 Technical 80-885942

Finland Orders 0800-914416 Fax 0800-914415 Technical 0800-914418

France Orders 01-60-920-926 Fax 01-60-920-925 Technical 81-60-920-930 Offers 01-60-920-928

Germany Orders 02103-29-12000 Fox 02103-29-22000 Technical 02103/29-12400

Hong Kong Orders 800 933 965 Fax 800 930 439 Technical 800 930 425

Ireland Orders 1800-555-049 Fax 1800-555-048 Technical 1800-555-061

Italy Orders 02-33430411 Fax 02-33430426 Technical 800-787980

Japan Telephone 03-5547-0811 Fox 03-5547-0818 Technical 03-5547-0811

Korea (South) Orders 1544 7145 Fax 1544 7146 Technical 1544 7145

Luxembourg - Orders 8002-2076 - Fax 8002-2073 - Technical 8002-2067

The Netherlands Orders 2890-0229592 Fax 0800-0229593 Technical 0800-0229602

Norway Orders 800-18859 Tax 800-18817 Technical 800-18712

Singapore Orders 65-67775366 Fax 65-67785177 Technical 65-67775366

Spain Orders 91-630-7050 Fax 91-630-5145 Technical 91-630-7050

Sweden Orders 020-790282 Fax 020-790582 Technical 020-798328

Switzerland Orders 055-254-22-11 Fax 055-254-22-13 Technical 055-254-22-12

UK * Orders 01293-422-911 * Fax 01293-422-922 * Technical 01293-422-999

USA Orders 800-426-8157 Fox 800-718-2056 Technical 800-DNA-PREP (800-362-7737)





Health Support

Queensland Forensic and Scientific Services

DNA Extraction from Animal Tissue using QIAGEN DNeasy Blood and Tissue Kit

1 Purpose and scope

This document describes the aseptic procedures required for sampling animal tissues prior to DNA extraction and the procedures utilised to extract and purity DNA using the QIAGEN DNeasy Blood and Tissue Kit. Manual and robotic extraction methods are described. These procedures can be applied to other animal tissues

2 Definitions

DNA

Deoxyribonucleic Acid

OGTR

Office of the Gene Technology Regulator

PC2

Physical containment Level 2

3 Principle

Advanced silica-gel-membrane technology allows rapid and efficient purification of total cellular DNA without organic extraction of ethanol precipitation. Samples are firstly lysed using proteinase K. Buffering conditions are then adjusted to allow selective binding of the DNA to the DNeasy membrane. Contaminants and enzyme inhibitors such as proteins and divalent cations pass through the membrane on centrifugation. Remaining contaminants are removed in two centrifuge wash steps. DNA is then eluted in water or buffer.

4 Reagents and equipment

4.1 Sampling in room 3155 Equipment

Electronic balance

Gloves, cutting board, foil, sterile scalpels, sterile disposable forceps, inoculation loops, plastic disposable petri dishes

Weighing trays to cutting small pieces of tissue,

Sterile 70mL containers for sample storage

Sterile 1.5mL and 2.0mL Eppendorf tubes

4.2 DNA extraction in room 3155 Equipment

Fume Extraction Cabinet

Heater block 37°C – 90°C

Vortex

Microcentrifuge capable of 20,000 x g

Micropipettes for volumes between 10µL and 1000µL

Filter barrier tips to fit micropipettes

Clean unused 1.5mL and 2.0mL Eppendorf

Collection tubes and spins columns supplied in kit





Inoculating loops

4.3 Reagents

Buffer ATL

Buffer AL

Buffer AW1 All reagents included in DNeasy Blood and Tissue Kit

Buffer AW2

Buffer AE

Proteinase K

Ethanol (96-100%)

When starting a new DNeasy Blood and Tissue Kit, buffers AW1 and AW2 must be diluted with ethanol and labelled accordingly. Refer to the DNeasy Blood and Tissue Kit Handbook for details.

5 Safety

When cutting whole fish wear safety goggles.

When working with chemicals always wear a suitable laboratory coat, disposable gloves and protective goggles.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Buffers AL and AW1 contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially harmful infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Buffer AL and Buffer AW1 contains guanidine hypochlorite: irritant.

Risk and safety phrases: R22-36/38, \$13-26/-36-46

Proteinase K: sensitizer, irritant.

Risk and safety phrases: R36/37/38442/43, S23-24-26-36/37

6 Sampling and sample preparation

6.1 Sampling of tissues for manual method (Room 3155)

- 6.1.1 Put on gown and fresh gloves. If cutting a whole fish wear safety goggles.
- 6.1.2 Prepare cutting board with fresh foil
- 6.1.3 Assemble sterile disposable scalpel, sterile disposable forceps, inoculating loop and plastic petri dish for cutting small pieces of tissue.
- 6.1.4 Label sterile 1.5mL Eppendorf tube and two sterile 70mL screw-capped containers.
- 6.1.5 Place the labelled Eppendorf tube in a container on the weighing pan and tare balance.
- 6.1.6 Dissect a sample from the centre of the fillet to avoid any possible contamination of the exterior of the fillet by previous handling (when a fillet has been received for analysis).
- 6.1.7 Place a small piece of tissue in the weighing tray (on foil-covered board) and use this tray to cut the piece of tissue into tiny pieces. The amount to be weighed out is 22-25mg and is to be cut into at least three pieces.



- 6.1.8 Use forceps to place tissue in labelled Eppendorf tube and use inoculating loop if necessary to enable manipulation of small pieces of tissue in tube when adjusting the amount in the tube.
- 6.1.9 Weigh 22-25mg tissue in labelled Eppendorf tube. It is important not to exceed 25mg of tissue.
- 6.1.10 Place approximately 15g of tissue in a screw-capped container for follow-up analysis and approximately 2g in a second screw-capped container for storage for DNA analysis.
- 6.1.11 Discard used scalpels, inoculating loops and forceps in a sharps container
- 6.1.12 Discard weighing tray foil and gloves in biohazard bag.
- 6.1.13 Wipe cutting board with 70% ethanol.
- 6.1.14 Use new gloves, foil, cutting tray, forceps, scalpel and inoculating loop for each sample.
- 6.1.15 Proceed with analysis or store weighed sample in freezer till ready to proceed with analysis. Place labelled screw-capped containers of samples in freezer.

6.2 Sampling of tissues for robotic method

6.2.1 Tissue sampling is performed in the same way as described in section 8.1 with the acceptation that approximately 300mg of tissue will be used and placed in a clean sterile unused 2.0mL Eppendorf tube appropriately labelled with the sample number.

7 Procedure

7.1 DNA Extraction in Room 3155 - Manual Method

NOTE: Room 3155 has OGTR PC2 certification. All operators using this room are required to be trained in the procedures necessary for compliance with this certification and signed records of the training are kept for inspection. Refer to Document QSP20036 Room 3155 Procedures/Operations Manual OGTR PC2 Compliance.

- 7.1.1 Pre-heat the heating block to 65°C.
- 7.1.2 Prepare a working list of samples to be extracted. (see Meat Sensimix PCR v4.xls)
- 7.1.3 Add 180µL Buffer ATL to 1.5mL Eppendorf tube containing 25mg tissue.
- 7.1.4 Add 20µL proteinase K, mix by vortexing (pulse vortexing 5-10 seconds) and incubate in heater block at 65°C until the tissue is completely lysed. Lysis is usually complete in 1-3 hours. Refer to DNeasy Blood and Tissue Handbook for more details.
- 7.1.5 Perform an RNase treatment of the sample by adding 5uL of RNase A post incubation and incubate for 2 minutes.
- 7.1.6 Vortex for 15 seconds, followed by a pulse centrifugation.
- 7.1.7 Add 200µL Buffer AL to the sample, mix thoroughly by vortexing, and incubate at 65°C for 10 minutes on the heating block.
- 7.1.8 Add 200µL ethanol (96-100%) to the sample, and mix thoroughly by vortexing.
- 7.1.9 Label a DNeasy Mini Spin Column and place in a 2mL collection tube (provided).







Figure 1: DNeasy mini spin column

- 7.1.10 Pipette the mixture from step 8.2.10 into the DNeasy Mini Spin Column.
- 7.1.11 Centrifuge at \geq 6,000 x g for one minute.
- 7.1.12 Discard flow-through and collection tube.
- 7.1.13 Place the DNeasy Mini Spin Column in a new 2ml collection tube (provided).
- 7.1.14 Add 500µL Buffer AW1, and centrifuge for one minute at \geq 6,000 x g.
- 7.1.15 Discard flow-through and collection tube
- 7.1.16 Place the DNeasy Mini Spin Columnina 2mL collection tube (provided).
- 7.1.17 Add 500µL Buffer AW2 and centrifuge for 3 minutes at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.
- 7.1.18 Place the DNeasy Mini Spin Column in a labelled 1.5mL Eppendorf tube.
- 7.1.19 Pipette 100µL Buffer AE directly onto the DNeasy membrane. Refer to DNeasy Blood and Tissue Kit Handbook for optional volume of Buffer AE and relationship to final DNA concentration and overall yield.
- 7.1.20 Incubate at room temperature for one minute.
- 7.1.21 Centrifuge for one minute at 6,000 x g to elute. When placing DNeasy Mini Spin Column and Eppendorf tube in the centrifuge be careful to bend the Eppendorf lid back so that it lies almost horizontal in the centrifuge. Place the centrifuge safety cover over the tubes before closing the centrifuge lid to ensure the Eppendorf lid do not snap off during centrifugation.





Figure 2: Placement of Eppendorf tubes in centrifuge



- 7.1.22 Discard DNeasy Mini Spin Column and close Eppendorf tube.
- 7.1.23 Estimate the concentration and purity of DNA by measuring the absorbance at 260nm wavelength on the Nanodrop 2000C.
- 7.1.24 Proceed with further analysis or store DNA extract as required

Note: There are more detailed notes in the DNeasy Blood and Tissue Kit Handbook on topics including amount of starting material, volume of Buffer AE, incomplete lysis and troubleshooting.

7.2 DNA Extraction - Robotic Method

- 7.2.1 Pre-heat the heating block to 65°C
- 7.2.2 A working list of samples to be extracted will be prepared prior to extraction. (See Template Glenn Current SensiFast PCR V2.xis)
- 7.2.3 Heat Buffer ATL in microwave for 15 seconds to dissolve any SDS
- 7.2.4 Add 600µL Buffer ATL to a 2.0 mL Eppendorf tube containing ~ 300mg of tissue
- 7.2.5 Add 80µL Proteinase K, mix by vortexing (pulse vortexing 5-10 seconds) and incubate on heater block at 65°C until the tissue is completely lysed (Figure 3). Lysis is usually complete in 4-24 hours depending on the food matrix. Refer to DNeasy Blood and Tissue Kit Handbook for further details



Figure 3: Heating Block containing samples for tissue lysis



- 8. Disposal Slot for Tips
- 9. Robotic Arm

Figure 4: Layout of the QIACube for DNA Extraction

- 7.2.14 Select appropriate extraction protocol on the QIAcube and follow all prompts. The robot will perform a self-check to ensure the platform is correctly loaded and that an adequate number of tips and volumes of buffers for the successful completion of the extraction have been loaded. Watch and wait while the robot does the self-check in case you have to replenish buffers and tips.
- 7.2.15 Once the robotic extraction is complete, unload newly extracted gDNA tubes from robot being very careful to keep the 1.5mL DNA extraction Eppendorf tubes and discard the used rotor adapters, columns and 2mL Eppendorf tubes (Figure 6).

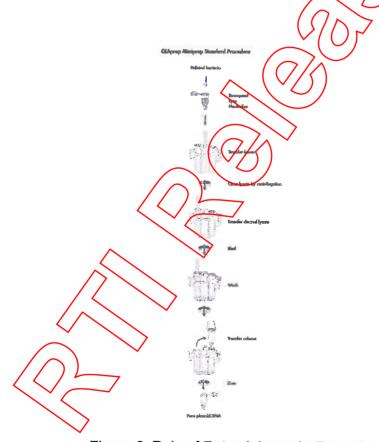


Figure 6: Role of Rotor Adapter in Extraction Method



- 7.2.16 Mix each gDNA extract and pulse spin the microfuge tubes to ensure contents are drawn to the bottom of the tubes
- 7.2.17 Empty the robot waste tip container and place the lids back on the buffer reagent bottles inside the QIAcube robot. Turn off the robot.
- 7.2.18 Estimate the concentration and purity of DNA by measuring the absorbance at 260nm wavelength on the Nanodrop 2000C

7.3 Spectrophotometry of extracted DNA using the NanoDrop 2000C

7.3.1 Turn computer, computer screen and NanoDrop instrument on at the power source.



Figure 7: NanoDrop 2000C Workstation

7.3.2 Thoroughly clean the Nano Prop platform (Figure 8) with Kimwipe lint free tissue. Discard tissue immediately into pathwaste bin.



Figure 8: NanoDrop 2000C instrument



- 7.3.3 Open NanoDrop 2000C program by clicking on the desktop shortcut. Follow prompts for instrument initialisation (no water is needed for initialisation)
- 7.3.4 Select "Nucleic Acid" button.
- 7.3.5 Pop-up window appears and asks whether you would like to load last workbook and append new data to it select "No"
- 7.3.6 Routine wavelength verification now occurs. The NanoDrop is ready to analyse the DNA samples. Discard all future used tips into the pathwaste tip container.
- 7.3.7 Clean NanoDrop platform and arm thoroughly with Kimwipe tissue. Discard tissue.
- 7.3.8 Before commencing blank measurement, check to see if correct sample type is selected (*Type-50* is double stranded DNA, *Type-30* is single stranded DNA/RNA).
- 7.3.9 Blank with AE Buffer (or with the solution used to resuspend your DNA/RNA) by applying 1-2uL to the platform and lower measurement arm. Select "Blank". Once blank measurement has completed, clean platform and arm thoroughly with Kimwipe tissue. Discard tissue.
- 7.3.10 Start measuring samples by applying 1-2ut to the platform and lower arm.

 Type in the name of sample (or sample code) in the Sample ID box. Click

 Measure. Clean platform and arm thoroughly between each measurement

 with Kimwipe tissue making sure the kimwipe tissue is discarded each time.
- 7.3.11 Once the first sample has been measured, a pop-up window will appear asking you to enter a workbook file name. Complete this task and save into your *Reports* folder on C drive. To find the reports, go to the desktop and select NanoDrop reports sportcut icon.
- 7.3.12 Repeat Step 9 for all samples. Successive sample measurements will be appended to your saved workbook in your *Reports* folder.
- 7.3.13 If a measurement error occurs (e.g. air bubble, misnamed sample ID etc...) repeat sample application and measurement. Sample errors can be removed from the final Excel report. Note: Sample errors cannot be removed in NarraDrop software.
- 7.3.14 To print measurement report:
 - Go/to Reports box
 - Select Configuration tab to make sure report layout has all the column headings you desire.
 - select Export and save file as *.xml in your reports folder. File can be opened in excel and Save As to a .xl file.
 - Alternatively, go to print tab. This generates a print preview and if the computer detects the wireless printer, hit Print.
 - If you need to perform more measurements, select Measure Nucleic Acid box again or finish by selecting Home box and closing the programme.

8 Validation

Refer to DNeasy Blood and Tissue Kit handbook.



9 Quality assurance/acceptance criteria

Refer to DiNeasy Blood and Tissue Kit handbook.

10 References

- DNeasy Blood and Tissue Handbook which accompanies the QIAGEN DNeasy Blood and Tissue Kit
- QSP <u>20036</u> Room 3155 Procedures/Operations Manual OGTR PC2 Compliance

11 Associated documents

QIS: DNA Extraction Training Module

QIS: Guidelines for the collection of fish and crustacean samples

12 Amendment history

Date	Author/s	\vee /() [Amendments
1 September 2006	Annette Badde	ey/Glenk G	raham	First Issue
Date	Updated by	0/1		Amendments
13 Oct 2014	G. Graham, C Wynwood	Lange and	S.	Consolidation of protocol to 3155 and QIS2 Migration. Inclusion of QIAcube robotics. Header/Footer changes General editing and tidy up of text addition of new information and new equipment.
	1 September 2006 Date	1 September 2006 Annette Badde Date Updated by 13 Oct 2014 G. Graham, C	1 September 2006 Annette Baddeley/Glank G Date Updated by 13 Oct 2014 G. Graham, C. Lange and S	1 September 2006 Annette Baddeley/Glank Graham Date Updated by 13 Oct 2014 G. Graham, C. Lange and S.

13 Appendices

1 Appendix A

QIAcube Protocol Sheet

Appendix B

QIAcube Loading Chart



Appendix A

QIACUbe Protocol Sheet

General Information (April 2010)

Application	DNA
KGI	Dt leasy® Blood & Tissue Kit
Sample material	Tissues and rodent tails
Short protocol name	Standard
Version	
Full protocol name	Purification of total DHA from animal tissues
Editable parameters	
Required QIAcube ⁸ software versions	Firmware version FIW-50-001 - J FW MB.hex and PLC program version FIW-50-002-G_PLC_MB.prs or higher; available from the QlAcube Web Porto

Shaker

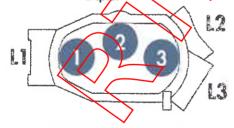
Material	In general, we recommend using 25 mg animal tissue. For rodent tail, use one (rat) or two (mouse) 0.4-0 6 cm lengths of tail.
	Samples must be digested with proteinase K in Buffer ATL before placing on the shaker (see "Comments" on the next page).
Vessel	2 ml safe-lock microcentrifuge pales"
Adapter	Shaker adapter for 2 ml microcentrituge tubes (marked with "2")

^{*}Sample Tubes RB, 2 ml (cat. no. 99038/1); see whos alianen.cam/MyQlAaube.

Disposable Tips

Disposable Filter-Tips, $1000\,\mu l$ Disposable Filter-Tips, $1000\,\mu l$ wide-bore

Rotor Adapter



Reagent Bottle Rack

		· · · · · · · · · · · · · · · · · · ·
Rack labeling	strip Dile	osy Tissue

1 – 2 Buffer AL 3 100% ethanol 4 Buffer AW1 5 Buffer AW2 6 Buffer AE	Position	Reagent
3 100% ethonol 4 Buffer AW1 5 Buffer AW2	ì	_
4 Buffer AW1 5 Buffer AW2	2	Buffer Al.
5 Suffer AW2	3	100% ethanol
	4	Buffer AW1
6 Buffer AE	5	Buffer AW2
	6	Buffer AE

Labveare	Lid position
Dt leasy Mini spin column	Lì
Bert	-
1.5 ml collection tube [†]	L3
	Dt leasy Mini spin column



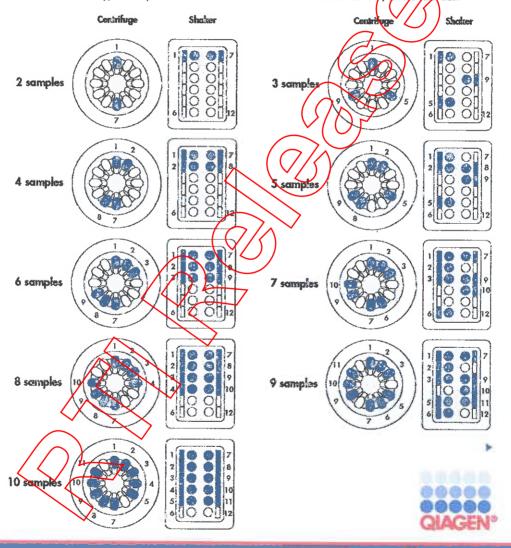


[†] Sorsiedt, Micro lube 1.5 ml, Safety Cap (see www.sarstedt.com).

Appendix B

GlAcube® loading chart

This Technical Information describes how to correctly load the QIAcube centrifuge and shaker if fewer than 12 samples are to be processed in a protocol run (see pictures below). When loading the shaker with samples in 2 ml microcentrifuge tubes, place tube lids into the slots at the edge of the shaker adapter. If the protocol requires the use of 2 ml screw-cap tubes, place Shaker Rack Plugs into the slots at the edge of the shaker adapter. Please note, I or 11 samples cannot be processed. For information about the type of sample and collection tubes that must be used with the QIAcube, see the other side.







HealthSupport Queensland Forensic and Scientific Services

Real-Time PCR of Mitochondrial Genes for Terrestrial Mammalian (Meat) Speciation

1 Purpose and scope

This document describes the procedures for amplification of a pertion of the mitochondrial cytochrome b gene of various terrestrial mammalian species. Comparison with certified reference samples enables the specific separation of whole meat or processed meat products. GenBank, an international sequence database and a FSS collection of certified mitochondrial cytochrome b reference sequences are available for the development of PCR based assays.

2 Definitions

FSS Forensic and Scientific Services
PCR Polymerase Chain Reaction
dNTP Deoxy Nucleoside Triphosphate
Cyto Cytochrome b gene

PC2 Physical containment Level 2

TE Tris(hydroxymethyl)aminomethane/ethylene diamine tetra-acetic acid

3 Principle

The mitochondrial cytochrome b gene codes for an essential cellular respiratory enzyme, cytochrome b. Therefore, regions of the gene are conserved (folding/active sites etc) between species. In addition to these regions, there are intervening sequences which can accumulate mutations over a given time without deleteriously affecting the function of the gene. The rate of mutation of this gene is such that it can be used as a taxonomic tool.

Part of the mitochordrial cytochrome *b* gene has been sequenced for many vertebrates. A substantial sequence database exists, including mitochondrial cytochrome *b* sequences of many terrestrial mammal species. These sequences can be accessed on the international sequence database, GenBank. Population geneticists and molecular systematists continue to contribute to this database. Certified reference samples have also been acquired and sequenced by Food Chemistry FSS.

Following sampling, DNA extraction and quantitation, a portion of the mitochondrial cytochrome *b* gene is amplified. By designing primers in conserved regions flanking divergent regions, amplification of a broad range of species can be achieved. Through the judicious selection of primer sequences within each of the species Cytochrome b gene specific amplification can be achieved as a diagnostic for the presences of that species DNA in a sample or add mixture of samples.

Primers have been designed or adapted for meat species and have been found by FSS to amplify specific species.



Detection of PCR products by gel electrophoresis is the traditional method for identification of PCR amplicons. However a new method of detection is now available and is being used widely to develop highly specific PCR amplification that requires no post-PCR electrophoresis and avoids the issues of cross contamination by detecting amplification in a closed PCR tube. This is known as Real Time PCR and utilizes fluorescent intercalating dyes to detect the accumulation of PCR product in a closed tube system. Subsequent High Resolution Melt (HRM) analysis of PCR amplicons is performed to establish the identity of amplified DNA fragments. HRM utilises the thermodynamic properties of double stranded DNA to detect the temperature at which the double strand dissociate or melt into single strands of DNA as the assay is incrementally heated to high temperatures. The process of melting DNA releases accumulated intercalated fluorescent dyes, allowing an instrument to detect the temperature with very high precision the point of dissociation. This dissociation temperature is very diagnostic with DNA amplicons having differing DNA sequences and so differing DNA melt temperatures. Instruments can detect a single nucleotide variation using this method.

4 Reagents and equipment

4.1 Reagents

Sterile distilled water (DNA free)
Primer Stocks
TE Buffer pH 8.0
Eva-Green Dye

4.2 Equipment

NanoDrop 2000 Spectrophotometer QIAGEN Rotor Gene Q 5 Plex Centrifuge with fixed angle rotor Microcentrifuge

5 Safety

Sample manipulation prior to DNA extraction should be performed in a PC2 laboratory.

There is no reagent safety issues associated with this test. Refer to individual MSDS documents for information on reagents. Refer to Chem Alert http://qheps.health.yld.gov.au/safety/hazards/chemalert.htm for information on chemistries used.

General Jaboratory safety procedures should also be followed

6 Sampling and sample preparation

Proceed according to SOP ""DNA Extraction from Seafood and Meat Tissue using QIAGEN DNeasy Blood and Tissue Kit 25332V5".

7 Procedure

7.1 Primers

When attempting to amplify an unknown mammalian species, it is necessary to utilise sets of primers as listed below:







In laminar flow cabinet (Room 2121), dilute primers to a stock primer concentration of 200pmoles/µL using TE buffer pH 8.0. Use the *Primer* and *Probe* calculator spreadsheet to calculate the stock dilutions.

Further dilute stock primers to a working dilution of 25pmoles/uL in DNA free sterile water. A dilution of DNA to 25pmoles/µL is calculated by dividing the DNA concentration by 25 expressed as a ratio. e.g. 187.2/25 = 1:7.5, therefore dilute DNA 10µL to 65µL.

7.2 Mastermix

The following mastermix (Table 2) is made up in the laminar flow cabinet in Room 2121. The amount of mastermix made is dependent on the number of samples being run. It is advisable to make mastermix for 10% more samples to allow for transfer losses.

The mastermix is dispensed in 19yL)lots into PCR tubes followed 1uL of the controls (Table 3) and samples. Final volume per reaction is 25uL.

Table 2: Mastermix Recipe

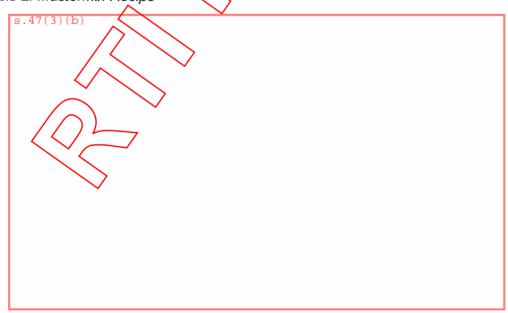
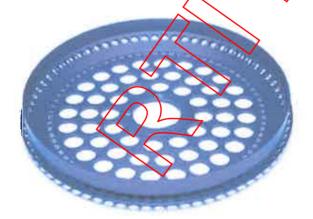


Table 3: Controls for real-time PCR (Meat)

Name	Species	Name	Species
Negative control	AE Buffer	Negative control	AE Buffer
Negative control	Water	Negative control	Water
Low Positive Control	Chicken	Low Positive Control	Pork
Positive control	Chicken	Positive control	Pork
Negative control	AE Buffer	Negative control	AE Buffer
Negative control	Water	Negative control	Water
Low Positive Control	Beef	Low Positive Control	Hørse
Positive control	Beef	Positive control	Horse
Negative control	AE Buffer	Negative control	AE Buffer
Negative control	Water	Negative control	Water
Low Positive Control	Sheep	Low Positive Control	Kangaroo
Positive control	Sheep	Positive control	kangaroo
Low Positive Control	Deer <	Environmental Control	AE Buffer
Positive control	Deer		
Negative control	AE Buffer		
Negative control	Water	(3)	

7.3 Rotor-Gene Q

The PCR tubes are then transferred to the Rotor-Gene Q ring (See picture 1).



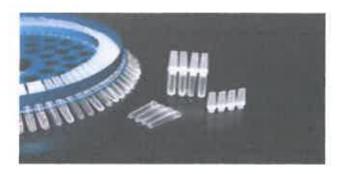


Figure 1: Rotor-Gene Q Ring and PCR tubes

Samples are then transferred to the Rotor-Gene Q detection unit for thermal cycling and detection. See Table 4.



Table 4: Cycling Conditions for Rotor Gene Q



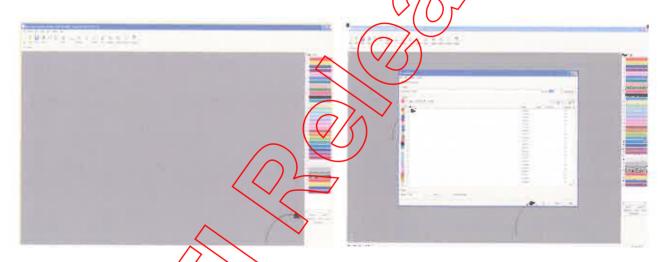
Total Run Time = ~72 minutes.

Rotor-Gene Q Operations:

1. Turn on computer and Rotor-Gene Q.

2. Open the "Glenn's SIM Meat Assay 72 Well" Template (APPENDIX A).

3. In the Rotor-Gene Q screen, click Edit Samples,



4. Type in the sample details, starting at position1, as per Table 5. Complete edit details for all meat species required. Click OK when completed.

Table 5: Sample details

Position:	Name:
1	C1 (sample)
2	C2 (sample)
3	Chicken Negative AE Buffer*
4	Chicken Negative Water*
5	Chicken High Positive
6	Chicken Low Positive
7	Blank Tube
8	B1 (sample)
9	B2 (sample)
10	Beef Negative AE Buffer*
11 (and so on)	Beef Negative Water*

^{*}addition of water or AE Buffer control will be determined by the sample diluent



C = Chicken

B = Beef

S = Sheep

P = Pork

K = Kangaroo (If required)

H = Horse (If required)

D = Deer (If required)

5. Select green Start button in menu bar to start run. Save run in folder under month. E.g. C://Desktop/Glenn's Most Excellent Stuff/Meat Assays

6. Once the run has completed, hit the Analysis button in the menu bar and select the

Quantitation tab. Double click on Cycling A. Green (Page 1),



7. Ensure that the linear scale is being viewed

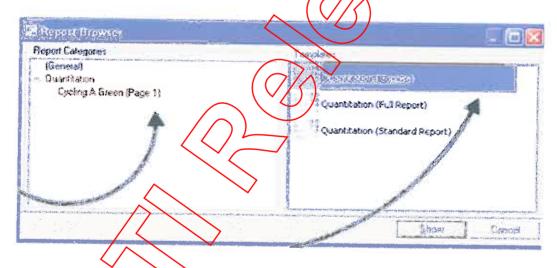


8. Hit Threshold button and adjust threshold to approximately 0.7 using the red line in the Quantitative Analysis box.





9. The Cycle to Threshold values will be displayed in the results box. A report should be generated by selecting the Reports tab from the menu bar and selecting Cycling A.Green (page 1) from the left menu. Double click on Quantitation (Concise) to show report.



8 Interpretation of test results

Positive Samples are defined as having a Cycle to Threshold (CT) value between 5 and 45 cycles.

Negative Samples: are defined as having a CT value greater than 45 cycles (not visible on the analysis).

9 Quality assurance/acceptance criteria

Results can be accepted when the positive, extraction and negative control samples have performed as expected i.e. the positive and extraction controls have a CT value between 5 and 40 and the negative sample has no CT value <u>AND</u> the environmental / No Template Control has no CT value.

10 Hazardous substances risk assessment

No hazardous substances are used in this assay.

11 References

QSP 20036 Room 3155 Procedures/Operations Manual OG/TR-RC2 Compliance

QIS25332V5 DNA Extraction from Seafood and Weat Tissue using QIAGEN DNeasy Blood and Tissue Kit

12 Associated documents

QIS Guidelines for the Collection and Species Identification of Fish and Crustacean Samples by Environmental Health Officers and Public Health Unit Staff.

13 Amendment history

Revision	Date	Author/s (//	Amendments
0	02/03/07	Glenn Graham	First Issue
Version	Date	Updated by	Amendments
2	10/10/14	Glenn Granam/ Sarah Wynwood	Migration to QIS2. Major changes Introduction of Real time PCR assay. Adaptation of original method to Rotor Gene technology Formatted to new Template and minor editorial changes



Page: 8 of 9



14 Appendices

1 Worksheet Template

14.1 Appendix A: Glenn's SIM Meat Assay 72 Well Worksheet Template

1	PCR Setup T	empiate		PCR assa	g order: C, B,	S,P	Date:		1	and the second second second		Personal Car ster.
2	PCR Mame: P	Reagent Preparati	ion	MB. Mas	termir vok	unes are i	nade for 2	Sef, react				
3	Date:						Cattle	Chicken	Sheep	Pig	Alberta I	PERK
4	Reagents		Satch No.	Cone.	Final Con	21	10	10	10	10	100	16
5	10 x PCR Sensin	nia		10 x	1x	12.50	125.00	125.00	125.00	125.00	1500	105.0
6	w ater			-	-	8,50	85.00	85.00	85 00	35 00	1000	15.0
7	MgCl ²			50m/V!	2.0mM	1.00	10.00	10.00	10.00	10.00	15.00	15.0
8	Primer f Standar	di .		25pM/uL	500 FMWuL	0.50		5.00	5.00	5.00	531	509
9 10	Primer 2 Chicke Primer 3 Cattle I			25pM/uL	500 AVAIL	0.50	-	5.00				
11	Primer 3a Cattle			25pM/uL 25pM/uL	500 RMAL 500 RMAL	0.50	5.00					
	Primer 4 Sheep	DN		25pM/std.	500 MVIral	0.50	5.00		5.00			
	Primer 5 Pig			25pM/uL	500 FMVuL	9.50		-		5.00	-	
	Primer 6 Horse					0.50	 			/	500	=
	Primer 7 Kangar	00				0.50						501
	EvaGreen dye					1.00	10 00	10.00	10.00	10 00	71600	-65
	Template			_	AMIN	1.00	of Marin	REINE	titud	-		
18	Yolume					25.00						
19	Dispense Ru	or Masternia •	23L of 50a	वध कारी	per 6.7ml	Rotores	e snierott	Me = 2000	volume s	ស្នេក្ខិក្រស្នេ	553/1	
20												
21	1	s and Results					Assan				ix used for as	5 ay
22	Sample No.	Client Number	Species	ng/uL	UL.	til.	Results	\ /		Not analy	ised (NA)	
-		1			Template ut		-/-	-/-				
24	1			50,0	2.0	0.0			Meat As	sag PCR	Program	
25	2			50.0	2.0	0.0		() ~	Temperature	Time (min:	se No. cycles	
26	3			50.0	2.0	0.0		\cup \setminus	95°C	9:00	1	
27	4			50.0	2.0	0.0	>/			4000		
		1					7/1					
28	5			50.0	2.0	0.0			94°C	0:10	35 for CBS	PKor
29	6			50.0	2.0	0.0			60°C	0:10	45 with H	
30	7			50.0	20	0.0			72°C	0:10		
11	8			50.0	2.0	0.0			120	V120		
-	9	-				$\overline{}$		=		,	141	
32				50.0	2.0	0.9			72°C	2.00	- 1	
))	10			50.0	(E.S)	/\d\0 \			4.0°C			
34	11			50.0	1 80/	00						
35	12			5008	2.0	0.0			Meat assa	nerénme	don	
36			/						1-16-06-000-0	perioning		
h.combeth	13		/	58.0	2.0	0.8						
17	14			50,0	5.0	0.0						
18	15		()	80.0	28/	0.0						
9	16			5030	2.0	0.0						
0	17			50.0	2.0	0.0			Assauning	aved on S	iver Fox or Li	nan
-195/a.s											st excellent s	
1	18			50.0	2.0	8.8			Assayst			
2	19			50.0	2.0	9.6						
3	20	/ >		50.0	2.0	0.0						
4	Sioassag Con	trols										
5		Negative Control	AE Buller	/	0.0	20						
وبب			· ·	∨ na	0.0	2.0			1			
6		Low Positive Sontr		0.5	2.0	0.0						
7		Positive Control	Chick on	50.0	2.0	0.8			L			
8		Negative Control	AE Buller	na	9.0	2.0						
9		Low Positive Contr	Beef	0.5	2.0	0.0						
0		Positive Control	7 Beef							Anna Service		
*****			THE ALLOW	50.0	2.0	0.0						
1		Megative Control	AE Eulifer	na	0.0	2.0						
2		Low Positive Contr	Sheep	0.5	20	0.0						
3		Positive Control	Sheep	50.0	2.0	0.0						
4		Negative Control	AE Buffer		0.0	2.0						
~ues	+			na								
5	+	Low Positive Contr	Pork	0.5	2.0	0.0						
6		Positive Control	Pork	50.0	2.0	0.0						
7		Magazine Committee	AF STORY	11000	100							
8		Dev From Lond	TOW	05	379	100						
						100						
9		FORMA (INC.)	-076	75	-25	35						
0		Mary Control	Miller		30	2.0						
7		Line Pagintin Com-	Assessed 1	85	120	AS						
1						_						
1		Pittern County	(Experience	1500	700							
		Environmental Con	Env	na	2.0	0.0						



na	AREA	Senal Number	Instrument Type	Type of Service Required					Status	Contact	Service period	Service Code	\$ Cost of Se	rvice	F
WAINTENING RATIONS.		(QIAcube	5 Day QN_01	48hr QN_02	24hr QN_03		No Svc							
100	PCR SUITE	11444	QlAcube	Х					Expired	Leon Scott	1/03/2014 - 28/02/2015	9240835	\$ 2,3	96.00	\$
JAN I	MICROBIOLOGY-MDU	7366	QIAcube	Х					Agreement	Cheryl Bletchly	1/03/2014 - 28/02/2015	9240835	Covered		
WAI		Q	Axtractor	5 Day QN 04	48hr QN 05	24hr QN_06	PipSvc QN_07	No Svc							
63	MICROBIOLOGY-MDU	5279	QIAxtractor	X					Agreement	Cheryl Bletchly	1/03/2014 - 28/02/2015	9241768	Covered		\$
On	MICROBIOLOGY-MDU	5447	QlAxtractor		Х				Agreement	Cheryl Bletchly	1/03/2014 - 28/02/2015	9241769	Covered		\$
7 +	MICROBIOLOGY-MDU	5448	QlAxtractor		Х				Agreement	Cheryl Bletchly	1/03/2014 - 28/02/2015	9241769	Covered		\$
18	TSV-MIC-VIROLOGY	2656	QiAxractor		Х				Agreement	Fleur Francis	1/03/2014 - 28/02/2015	9241769	Covered		\$
OCHM,			EZ1	5 Day QN 08	48hr QN_09	24hr QN_10		No Svc							
W	TISSUE TYPING	0502F0704	EZ1					Х	Expired	Alycia-Thornton	NA NA				\$
Q	TISSUE TYPING	L126A0837	EZ1 Advanced XL			Х			Expired	Alycia Thernton	1/03/2014 - 28/02/2015	9239959	\$ 7,66	37.00	\$
B		QIA	symphony	5 Day QN 11	48hr QN_12	24hr QN_13		No Svc							
~	MICROBIOLOGY-MDU	6993	QlAsymphony SP		Х		<		Expired	Cheryl Bletchly	1/03/2014 - 28/02/2015	9241207	\$ 12,51	9.00	\$
S	PCR SUITE	7749	QIAsymphony SP		Х				Expired	Leon Scott	1/03/2014 - 28/02/2015	9241207	\$ 12 ,51	9.00	\$.
5		Liquid Handling (QlAgility, CAS-1200, 1200N, 4200)		5 Day QN_17	48hr QN_18	24hr QN 19	PipSvc QN_20	No Svc							
ASREGMENT	MICROBIOLOGY-MOL MICRO	3372	QIAgility					5)	Expires 12/10/15	Haakon Bergh	NA				\$
Ž.	MICROBIOLOGY-MDU	A100302	CAS-1200	Х					Expired	Cheryl Bletchly	1/03/2014 - 28/02/2015	9241762	\$ 3,52	9.00	\$
X	MICROBIOLOGY-MDU	A100303	CAS-1200		X				Expired	Cheryl Bletchly	1/03/2014 - 28/02/2015	9241763	\$ 4,23	5.00	\$
4	MICROBIOLOGY-MDU	A060717	CAS-4200			Х			Expired	Cheryl Bletchly	1/03/2014 - 28/02/2015	9241764	\$ 5,35	8.00	\$
	MICROBIOLOGY-MDU	A070713	CAS-1200	X					Expired	Cheryl Bletchly	1/03/2014 - 28/02/2015	9241762	\$ 3,52	9.00	\$
7	MICROBIOLOGY-MDU	A040604	CAS-1200	X					Expired	Cheryl Bletchly	1/03/2014 - 28/02/2015	9241762	\$ 3,52	9.00	\$
Z	TSV-MIC-VIROLOGY	A040808	CAS-1200		Х				Expired	Fleur Francis	1/03/2014 - 28/02/2015	9241763	\$ 4,23	5.00	\$
12	TSV-MIC-VIROLOGY	A040810	OAS-1200	>	Х				Expired	Fleur Francis	1/03/2014 - 28/02/2015	9241763	\$ 4,23	5.00	\$
N/6	PCR SUITE	A060716	CAS-1200		X				Expired	Leon Scott	1/03/2014 - 28/02/2015	9241763	\$ 4,23	5.00	\$
WAIN	PCR SUITE	A120307	CAS-1200N					Х	Expired	Leon Scott	NA				\$
		Rol	tor-Gene	Ann Insp QN_21	Full Agr QN_22	Prem Agr QN_23		No Svc							
پ [MICROBIOLOGY-MDU	H060417	ROTOR-GFNE 3000	N/A	N/A	1/A			Expired	Cheryl Bletchily					\$
2002	MICROBIOLOGY-MDU	R070414	ROTUR-GENE 3000	NΆ	N/A	MA			Existed	Cheryl Stetohiy					\$
0	MICROBIOLOGY-MDU	R060731	ROTOR-GENE 6000		Х				Expired	Cheryl Bletchly	1/03/2014 - 28/02/2015	9241781	\$ 3,30	9.00	_
	MICROBIOLOGY-MDU	ER060744	ROTOR-GENE 6000		Х				Expired	Cheryl Bletchly	1/03/2014 - 28/02/2015	9241781		9.00	
		320	RG-0/5-Piex HRM		Х	RTI	Document	No. 1	Expires 17/02/2014	Cheryl Bletchly	17/02/2014 - 17/02/2015	9241781		9.00	

AREA	Serial Number Instrument Type		Type of Service Required					Warranty Status	Contact			
·		QiAcube	5 Day QN_01	48hr QN_02	24hr QN_03	PM service	No Svc	Gialds				
FSS-FOOD CHEMISTRY	11538	QIAcube				9241225		Expires 19/4/2014	Glenn Graham	19/4/2014 - 19/4/2015	9241225	\$ 2,660.00
	Xtractory	gene (CAS-1820)	5 Day QN 04	48hr QN_05	24hr QN 06	PipSvc QN_07	No Svc	131412014				
FSS-MICROBIOLOGY	A060715	CAS-1820					X	Expired	Arny Jennison	NA		
		EZ1	5 Day QN 08	48hr QN_09	24hr QN_10		No Svc					
F8S-VIROLOGY	0507F0925	EZ1	Х					Expired	Frederick Moore	1/03/2014 - 28/02/2015	9238850	\$ 4,672.0
FSS-VIROLOGY	L093A0017	EZ1 Advanced XL		Х				Expired	Frederick Moore	1/03/2014 - 28/02/2015	9237721	\$ 5,513.0
FSS-LEPTOSPIROSIS	L101A0315	EZ1 Advanced XL	Х					Expires 24/3/2014	Scott Craig	24/03/2014 - 24/03/2015	9238850	\$ 4,672.
		Jniversal	5 Day QN 14	48hr QN_15	24hr QN_16		No Svc	24/0/2014				
FSS-VIROLOGY	3943	BioRobot UNIVERSAL	Х					Expired	Frederick Moore	1/03/2014 - 28/02/2015	9239571	\$ 20,126.
FSS-VIROLOGY	4105	BioRobot UNIVERSAL	Х					Expres 23/03/2013	Frederick Moore	1/03/2014 - 28/02/2015	9239571	\$ 20,126.
	Liquid Handling (CAS-1200, 1200N, 4200)	5 Day QN 17	48hr QN_18	24hr QN_19	PipSvc QN_20	No Svc	20/30/20/3				
FSS-VIROLOGY	A050715	CAS-1200	Х		411		111	Expired	Frederick Moore	1/03/2014 - 28/02/2015	9241762	\$ 3,529.
FSS-VIROLOGY	A060708	CAS-1200	Х				7//	Expired	Frederick Moore	1/03/2014 - 28/02/2015	9241762	\$ 3,529.
FSS-VIROLOGY	A070711	CAS-4200	Х					Expired	Frederick Moore	1/03/2014 - 28/02/2015	9241762	\$ 3,529.
FSS-VIROLOGY	A070712	CAS-4200	Х			1		Expired	Frederick Moore	1/03/2014 - 28/02/2015	9241762	\$ 3,529.0
FSS-MICROBIOLOGY	A120607	CAS-1200N	X		1	×		Expired	Amy Jennison	1/03/2014 - 28/02/2015	41762 + 92388	\$ 5,207.
FSS-MICROBIOLOGY	A060723	CAS-4800					Х	Expired	Amy Jennison	NA		
FSS-MICROBIOLOGY	A060725	CAS-1200					PM Service 9241776	Expired	Glenn Graham	1/03/2014 - 28/02/2015	9238838	\$ 1,678.
	R	otor-Genta	Ann. Insp QN_21	Full Agr QN_22	Prem Agr QN_23		No Svc					
FSS-VIROLOGY	R070749	ROTOR-GENE 6000			Х			Expired	Frederick Moore	1/03/2014 - 28/02/2015	9242698	\$ 4,990.0
FSS-VIROLOGY	R110648	ROTOR-GENE 6000			Х			Expired	Frederick Moore	1/03/2014 - 28/02/2015	9242698	\$ 4,990.0
FSS-VIROLOGY	R020916	RG-Q 5-Plex HRM			Х			Expired	Frederick Moore	1/03/2014 - 28/02/2015	9242698	\$ 4,990.
FSS-VIROLOGY	R0810217	RG-Q 5-Plex HRM			Х			Expires 13/04/2014	Frederick Moore	13/04/14 - 13/04/15	9242698	\$ 4,990.
FSS-MICROBIOLOGY	R070761	ROTOR-GENE 6000					Х	Expired	Amy Jennison	NA		
FSS-MICROBIOLOGY	R0410161	RG-Q 5-Plex HRM	Х					Expires 31/05/2013	Glenn Graham	31/05/2014 - 31/05/2015	9241778	\$ 1,878.
FSS-FOOD CHEMISTRY	R0810206	RG-Q 5-Plex HRM	Х					Expires 19/4/2014	Glenn Graham	19/04/2014 - 19/04/2015	9241778	\$ 1,878.
	S/AR	QIAXON AI	Basic QN_24	Full QN_25	Full + PM QN_26		No Svc					
▎┍┑╏═╏╏╶ ╱╟	(6) ` ((Document	No.2	Expires	Frederick Moore	NA		



HealthSupport

Queensland

Forensic and Scientific Services

Room 3153 Procedures/Operations Manual OGTR PC2 Compliance

1 Scope

This document describes the operating procedures to be adhered to within room 3153 in order to ensure compliance with OGTR PC2 certification. All operators and staff intending to perform laboratory activities within Room 3153 of Public Health Microbiology Laboratory must first be trained in the procedures and requirements outlined in this document and signed records of this training are to be compiled and kept for inspection.

2 Safety

Safety procedures with respect to biological activities within room 3153 shall be in accordance with AS/NZS2243.3:2010 Australian/New Zealand Standard Safety in Laboratories: Microbiological Aspects and containment facilities.

Further, any additional requirements to AS/NZS2243.3:2010 as specified in the 'Guidelines for Certification of a Physical Containment Level 2 Laboratory' document will be adhered to in order to ensure compliance with the regulations.

General laboratory safety requirements under existing WH&S legislation also apply. Staff members are also required to have received FSS WH&S induction and sign-off prior to commencing any activities within the laboratory facilities.

3 Referenced documents

AS/NZS2243.3;2010 Australian/New Zealand Standard Safety in Laboratories: Microbiological Aspects and containment facilities
OGTR Guidelines for Certification of Physical Containment Level 2 Laboratory.
AS/NZS2252.4; "Biological safety cabinets - Installation and use".
OGTR Guidelines for the Transport, Storage and Disposal of GMOs

QIS 32659 — Public Health Microbiology PC2 Safety Manual QIS 31335 — SSBA/OGTR/DAFF Dedicated Autoclave Run

4 Definitions

OGTR - Office of the Gene Technology Regulator

PC2 - Physical Containment Level 2

GM - Genetically Modified

GMO - Genetically Modified Organism
IBC - Institutional Biosafety Committee

DIR - Dealings with a GMO Involving Intentional Release to the Environment





DNIR -Dealings with a GMO NOT Involving Intentional Release to the Environment

NLRD -Notifiable Low Risk Dealing with a GMO

5 **Principle**

Purpose of Certification

Certain 'dealings' with GMOs must be conducted within physical containment facilities. The purpose of certification is to satisfy the Regulator that the containment facility protects persons outside the facility from exposure to GMOs and prevents release of GMOs into the environment. The Regulator also imposes conditions of certification that require certain procedures to be followed to ensure the safety of people working inside containment facilities with GMOs.

Noncompliance with the Gene Technology Act is dealt with under the Criminal Code. The Gene Technology act stipulates that all organisations involved with GMO dealings must be accredited, must have access to an IBC and that any facilities where GMO dealings are conducted must be certified by the OGTR.

6 **Facility Manager**

The Chief Scientist, John Bates is the facility manager/Contact details are:

John Bates

Chief Scientist

Public Health Microbiology Laboratory

Communicable Disease

Queensland Health Forensic & Scientific Services

Ph: 61-7-32749101 Fax: 61-7-32749175 Mob: 0414747141

Email: john bates@health.gld.gov

7 **Authorised Persons**

While any dealings with GMOs are being conducted in the facility, the certification holder must ensure that access to the facility is restricted to authorised persons.

An authorised person is a person who has been trained in Behavioural requirements, has produced signed evidence of this training (captured in a training module document) and who has permission to enter the facility. Additional training will be rolled out in the form of questions etc if and when standards and guidelines are updated.

8 General requirements for a PC2 laboratory facility

General

- 1. Dealings in Room 3153 must not be at a level higher than PC2.
- 2. Must not produce more than 25 litres of liquid culture of GMOs in one vessel.

Facilities

- *The facility must be labelled with the following adhesive signs as supplied by the OGTR:
 - Physical Containment Level 2 (PC2) sign on the outside of facility access door(s):
 - biohazard symbol on the outside of facility access door(s); and





- ^{*}The facility must be a fully enclosable space contained within walls, doors, windows, floors and ceilings. Doors must remain closed during work.
- Walls, floors, ceilings and benches and furniture, including seating, must be smooth, impermeable to water, cleanable, and resistant to the cleaning agents and/or disinfectants used in the facility.
- 4 A wash basin must be provided for hand washing within the facility. The wash basin must be fitted with a basin mixer of the hands-free operation type.
- 5 Eyewash facilities (either a plumbed eyewash facility or single-use packs of sterile eye irrigation fluids) must be provided within the facility. Eyewash facilities must be used and maintained in accordance with the manufacturer's instructions.
- The facility must contain a pressure steam steriliser (autoclave) or have an autoclave that is accessible to facility users. If the autoclave is not located in the facility, it is preferable that it be located within the same building as the facility.
- 7 Designated storage or hanging provisions for protective clothing must be available within the facility.
- A supply of disinfectants suitable for the agents in use must be available in the facility. The disinfectants must be clearly labelled with the contents and, where necessary, the expiry date.
- 9 Open spaces between and under benches, cabinets and equipment must be accessible for cleaning.

Personal protective clothing and equipment

- 10 Protective clothing to protect the front part of the body must be worn by all persons performing procedures in the facility.
- 11 Closed footwear must be worn.
- 12 Gloves must be worn for work undertaken in a biological safety cabinet or when handling GMO bacteria which fit into the classification of Risk Group 2 organisms as described in AS/NZS 2243.3.
- 13 Protective clothing must be removed after completing laboratory procedures and before leaving the facility. This requirement does not apply if entering another containment facility, certified to PC2 by the Regulator, which is directly connected to the facility.

Containment equipment

- 14 If procedures that generate aerosols containing GMOs are to be performed in the facility, the facility must contain a biological safety cabinet, or other equipment specifically approved in writing by the Regulator that is designed to contain aerosols
- 15 Installation, use and decontamination of the biological safety cabinet must be in accordance with the requirements of AS/NZS2252.4: "Biological safety cabinets Installation and use".
- 16 The biological safety cabinet must be tested by a qualified person at least every 12 months as per AS 2252.1:2010 and 2252.2:2010. The cabinet must be labelled to show its test status.

Work Practices

17 *All requirements for a PC2 Laboratory Facility specified in the Certification Instrument issued by the Regulator must be complied with at all times, even if the work being performed in the facility involves organisms that are not GMOs unless

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- there is evidence that there is no GMO handling occurring at the time so there is no risk of cross contamination.
- ^{*}Windows must remain closed while laboratory procedures are in progress unless they are fitted with intact flyscreens. Facility doors must be closed when laboratory procedures are in progress.
- 19 All facility personnel must be trained in the requirements of the OGTR PC2 Laboratory Facility Guidelines. Only trained personnel are to clean contaminated equipment and surfaces, or handle hazardous material.
- Facility personnel must indicate to the certification holder that they fully understand their training in the OGTR requirements by signing a record of their training after completion. A record of those trained must be kept and made available if requested.
- 21 Any procedures that generate aerosols containing GMOs must be performed in a biological safety cabinet or other equipment designed to contain aerosols specifically approved in writing by the Regulator.
- 22 Any unintentional release of GMOs from the facility must be reported to the Regulator as soon as practicable.
- Work benches, surfaces and equipment where laboratory procedures have taken place must be decontaminated immediately after any spills and when laboratory procedures using GMOs are completed.
- 24 All work surfaces and equipment, in relevant areas of the facility, must be decontaminated before maintenance is carried out.
- 25 GMOs, organisms infected with GMOs, equipment or protective clothing contaminated with GMOs, and liquid and solid wastes containing GMOs, must be decontaminated in accordance with Section 3.1 of the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs.
- Where a pressure steam steriliser (autoclave) is used for decontamination, the waste and materials must be transported to the autoclave in a sealed secondary container. Organise ahead of time with the sterilisation group and ensure all details are recorded on the autoclave form QIS 3/335.
- ^{*}All GMOs, and waste potentially contaminated with GMOs, being transported out of the facility must be transported in accordance with the "Guidelines for the Transport, Storage and Disposal of GMOs".
- ^{*}GMOs or organisms infected with GMOs may be stored outside the facility in a storage unit (freezer, fridge, controlled temperature room or other controlled temperature container). The storage unit must be locked when not in use, unless access is restricted to the room or area where the storage unit is located, and have a biohazard symbol posted on it.
- ^{*}GMOs or organisms infected with GMOs being stored outside the facility must be double-contained. The primary container must be sealed and unbreakable. The primary container must be stored in an unbreakable secondary container and clearly labelled. In the case of a small storage unit such as a fridge, freezer or liquid nitrogen container, the secondary container may be the storage unit.
- Any spills that occur during storage outside the facility or when transferring to the storage unit, must be reported to the Regulator as soon as practicable. The spilt material and the area must be decontaminated.
- 31 All cultures must be clearly identified.
- 32 All cultures of fungi and other spore-dispersing organisms must be sealed during storage.
- 33 Eating, drinking, smoking, shaving and applying cosmetics are prohibited in the facility. Food or drink intended for human consumption must not be brought into or stored in the facility.
- 34 Long hair must be tied back or covered with a hair net to avoid contamination.
- 35 Mouth pipetting is prohibited in the facility.



- Only reading/writing material and computers essential to procedures performed within the facility are permitted on work benches where procedures are performed. Reading and writing material must not be used inside a biological safety cabinet. Where possible dedicated reading/writing areas should be provided and used.
- 37 Persons who have been performing procedures in the facility must wash or decontaminate their hands immediately before leaving the facility or before using any dedicated facility reading/writing areas.
- 38 The facility and equipment in the facility must be maintained so that the facility meets the containment requirements of these Guidelines.
- 39 Strategies must be in place to ensure that the facility is free of pests. A record of the program and dates of specific activities must be kept and made available if requested.

9 PHM specific procedures for working in Room 3153

9.1 Entry

If GMO work is occurring then access to room 3153 is limited to staff which have the authorisation and have signed a statement that they have read and understood this document, and have been issued with a valid DKS security key which allows entry to the PC2 area of the Public Health Microbiology Laboratory. Individuals who do not possess a valid key to access Public Health Microbiology must only be admitted to room 3153 under the supervision/authorization of a staff member who possesses a valid DKS key (examples of such scenarios may include for example periodic visits by contractors/repair personnel).

On entry a disposable lab coat shall be donned by those intending to perform procedures, and must be worn for the duration of those activities within the room. Lab coats shall not be removed from room 3153 except via inclusion in designated waste for autoclaving as described later in this document. Closed footwear must be worn. Gloves MUST be worn for work undertaken in this room.

Cultures should be extracted for nucleic acids in BSCII cabinets or to limit the potential for contamination in this small room.

9.2 Exit

Without exception, all persons who have been performing procedures (GMO related or not) within room 3153 must wash their hands using the basin fitted with a hands-free faucet before exiting the room.

Disposable laboratory soals must be removed prior to exiting the facility and either stored on the coat hooks or in the pigeon holes provided, or alternatively discarded into the GMO waste receptacle.

Gloves shall not be worn out of the room or taken out of room 3153 except via the defined waste management protocols detailed in this document.

** NOTE: The door to room 3153 MUST remain closed at all times **

10 Laboratory Activities in Room 3153

Reading and writing equipment (excluding marking pens provided explicitly for use within room 3153) are not to be placed inside a biological safety cabinet. Marking pens

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provided for use within room 3153 must not be removed from the facility except by way of waste handling as described below in point 9.

All GMO cultures must be labelled to indicate that they contain GMO organisms.

When not being manipulated (e.g. incubation, storage) all GMO related cultures are to be sealed with Nescofilm/Parafilm.

Work benches, surfaces and equipment where laboratory procedures have taken place must be decontaminated immediately after laboratory procedures using GMOs are completed. Decontamination is to be performed using 80% Ethanol in the wash bottles provided, followed by "Trigene", and then water. Any alternative chemical disinfection must be in accordance with Appendix F of AS/NZS 2243.3:2010, and any decontamination solution(s) must be labeled with the applicable expiry date(s).

11 Waste Handling for Room 3153

All GMO related waste shall be deposited into biohazard bags lining the lidded Perspex bin. Since the location of the autoclave is not within room 3153, procedures for transporting waste for autoclaving must adhere to transport requirements for GMOs discussed in section 13 below. Consequently all Petr disres are to be sealed with nescofilm/parafilm prior to discard into biohazard bags in the acrylic bin.

Biohazard bags containing GMO related waste for autoclaving shall be sealed with autoclave tape and placed into decontaminated plastic bins/eskies with lids which seal airtight, and then moved from room 3153 to the autoclaving facility. Gloves must be worn whilst transferring the biohazard bags into and out of the sealed bins/eskies. The bins/eskies must then be decontaminated at the autoclave facility before being returned to room 3153.

The procedure for dedicated autoclave runs from room 3153 is detailed in the QIS document 31335. Part A of the form "OGTR / AQIS Dedicated Autoclave Run" (found in the appendix of document 31335) must be completed and the form given to the Glassware and Sterilisation (G&S) staff. A suitable time for availability of a dedicated autoclave must be pre-arranged with G&S staff prior to transporting the waste to the autoclaving facilities. G&S staff will complete Part B of the form and return it to you, check and then file this form in the "Dedicated Autoclave Runs" folder/ring binder located on the bookshelf outside of room 3153.

Chemical waste stored in the dedicated sharp bin containers or recycled solvent bottles in the furne moods shall NOT be disposed by autoclaving. The sharp bins containing chemicals shall be sent by arrangement to Ace waste.

12 Maintenance—Cleaning

While the room is utilized for exempt dealings, the designated cleaning staff will clean the floor fortnightly by the normal method and sign the register that this has been performed. When the room is to be designated for GMO then a rostered PHM scientist for the room 3153 take the dedicated steel bucket with lid and fill partially with warm water and detergent. The dedicated broom shall be used to remove dropped plastics and dirt and place into the bag of the Perspex waste container. Following this, the floor shall be cleaned and the waste water autoclaved by use of the OGTR form.



The assigned book for floor cleaning, outside the OGTR room, shall be filled out by the officer

13 Specimen storage and transport

13.1 Storage

At this stage ALL GMOs (no exceptions) must only be stored within the registered facility, room 3153 and must be sealed. OGTR PC2 regulations do permit the storage of GMOs or organisms infected with GMOs externally to the facility provided they are doubly contained and stored within a locked storage unit which has a biohazard sign affixed. However, until space limitations arise in room 3153 all GMOs are to be stored only within the facility. This will avoid unnecessarv packaging/unpacking/decontamination which would otherwise be involved in the transport of GMOs requiring storage from room 3153 to an external storage facility. A sign shall be displayed on Refrigerators used for storage of GMOS/arto designating what is contained within, and where positioned. Boxes containing GMOs within the refrigerator shall be labelled accordingly.

13.2 Transport

Transport of GMOs shall be in accordance with "Guidelines for the Transport, Storage and Disposal of GMOs". Transport is defined as all movements of a GMO from a certified facility to ANY location OUTSIDE the certified facility. For the purposes of this document, facility refers to room 3153. In addition it is the responsibility of the authorised person in relation to the GMO to ensure that any third parties involved in the transport of a GMO (e.g. sub contractors/transport companies) are made aware of (and comply with) the conditions relating to the transport of GMOs.

Permission to transport a GMO is not automatic, permission for transportation of GMOs are defined by the classification of the GMO:

Exempt Dealings GMOs may be transported without approval from the Regulator

Notifiable low risk dealings with GMOs may be transported only after notification of the Regulator and only then if the conditions in the regulations are complied with

Dealings with GMOs on the GMO Register may be transported with compliance to any specific conditions noted on the GMO register

All other dealings with GMOs, if the GMO is not on the list of exemptions, not on the list of notifiable low risk dealings and not on the GMO register, you must not transport the GMO unless you have a licence from the regulator to do so.

Conditions related to transport of GM micro-organisms

The GM micro-organism must be wholly contained within a primary sealed container AND the primary sealed container must be packed in a secondary airtight sealed unbreakable container. Gloves must be worn whilst handling the primary container. Examples of a primary sealed container include: a petri dish sealed with parafilm or a sealed plastic tube.

The secondary unbreakable container must be labelled to indicate that it contains GM micro-organisms, and the label must include the telephone number of a person to contact should the package be damaged or lost.



Accounting procedures must be in place to ensure that the same number of containers sent is delivered. Telephone confirmation should be sort as a minimum

Following transport of the GMO, the primary sealed container and the secondary unbreakable container must be decontaminated to ensure that no residual GMO is retained, or alternatively the containers must be destroyed.

Conditions relating to compliance with relevant packaging and transport regulations

All relevant packaging and transport regulations must be complied with for the transport of GMOs including, where relevant:

International Air Transportation Association (ATA)/Dangerous Goods

Australia Post Dangerous Goods and Packaging Suide

The Australian Code for the Transport of pangerous Goods by Road and Rail

Local Public Health regulations for the transport of infectious wastes

Shipping and packaging for air transport must always be conducted by a current IATA certified staff member (currently Mary-Anne Burns).

14 Spills and/or unintentional GMO release

Spills within the facility will be dealt with according to procedures in QIS 32659 using the appropriate disinfection in accordance with Appendix F of AS/NZS 2243.3:2010. All PPE and clean up materials for disposal must be autoclaved as per Section 11.

Any unintentional release of GMOs or organisms containing GMOs must be reported to the regulator as soon as practicable

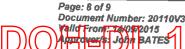
Any spills outside of room 3153 (e.g. during storage or transport) of GMOs or organisms containing GMOs must be reported to the regulator as soon as practicable. material and the area shall be immediately cleaned up utilising the procedures in QIS 32659...

Reporting of unintentional release and/or spills outside of room 3153 (e.g. during storage or transport) shall be accomplished by contacting the Chair of the FSS IBC (Currently Lee Smythe - 3274 9064), or their delegate, who will then handle the advisement of the OGTR.

Records will be kept by the Chair of the IBC relating to any external spill or other unintentional release of a GMO or organism containing a GMO.

15 Equipment repairs, maintenance, removal for servicing or decommissioning

All work surfaces in room 3153 and the relevant equipment requiring maintenance/repair must be decontaminated before any repairs/maintenance is carried This is the responsibility of the facility person who arranges the repairs or maintenance, and in their absence if the decontamination has not knowingly been performed prior to the arrival of the service personnel, the responsibility of ensuring the





decontamination procedures are undertaken (or have been undertaken) is transferred to a facility staff member (OGTR requirements trained) who oversees the admission of any repair/maintenance technicians entry into room 3153. Decontamination if not done prior needs to be completed before access is granted to room 3153.

Equipment which requires removal from room 3153 for servicing or decommissioning is to be decontaminated prior to such removal.

Decontamination shall be performed by wiping accessible internal and external surfaces of equipment with 80% ethanol followed by "Trigene" solution and then sterile water.

16 Pest control

Regular pest treatments are organized and recorded by facilities management of FSS.

17 Amendment history

Revision	Date	Author/s		Amendments
0	10 Sept 2004	S Byrne		First Issue
2	4 th April 2008	J Savill		Second issue
			(Ω)	New PC2 guidelines;
			<i>∨</i> /(Modifications to cleaning;
				Additions to training;
			(O/Δ)	Modification to operations
2	April 2008	QIS2	Migration	Headers and Footers changed to
		Project		new CaSS format. Amended
				Business references from QHSS
		$ (\alpha)$	\sim	to FSS, QHPSS to CaSS and
				QHPS to Pathology Queensland
3	August 2015	A Jennison		Updated and edited to reflect
	//			latest documentation and
				practices





HealthSupport

Oueensland Forensic and Scientific Services

Quality Management System Guide

Purpose

Quality systems certification (under ISO 9001) and laboratory technical accreditation (under ISO 17025, ISO 15189, ISO 17043 and ISO Guide 34) requires documentation of all policies and procedures that are critical to the proper functioning of Forensic and Scientific Services (FSS).

The purpose of this guide is to provide an "electronic index" to the principal documents in QIS that describe the quality management system as it applies to PSS Details of the accreditation, certification and approvals held by FSS are detailed in section 7. This section also details the exclusions for certification against ISO 9001.7

2 Scope

This guide is designed to be used by all staff at Forensic and Scientific Services, as well as clients and external assessors of the quality management system.

3 **Definitions**

Nil

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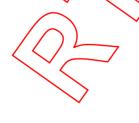
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4 Organisation overview

Forensic and Scientific Services is part of Community & Scientific Support, a service line of the Health Support Queensland (HSQ). This reporting relationship is available on the HSQ staff website.

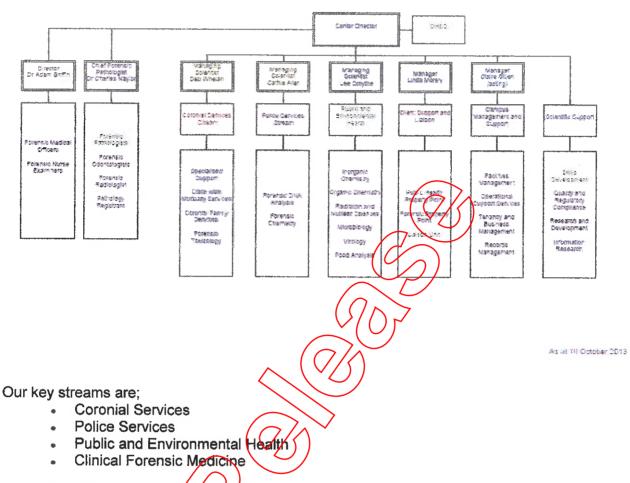
Forensic and Scientific Services (FSS) is Australia's most comprehensive forensic and public health science facility. We provide expert analysis, advice, teaching and research as a vital part of the government response to threats to public health and the environment, epidemics, civil emergencies, criminal investigations and coroners' inquiries into reportable deaths.

The Forensic and Scientific Services (FSS) organisational structure is below.



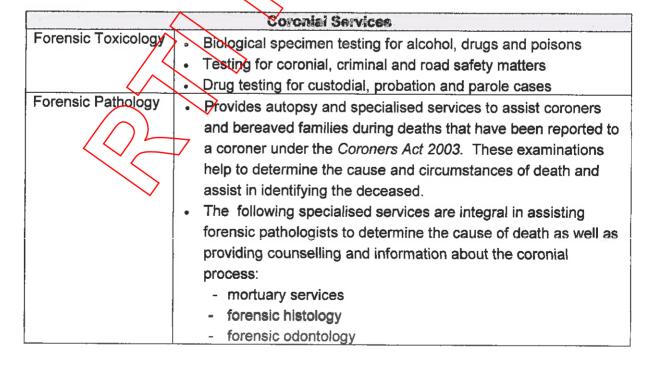






FSS Realignment to a Client Facing Model

The units within each stream, and the corresponding services provided by these units is detailed below;



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- forensic skeletal examination				
- coronial counselling service				
- coronial tissue donation				
- coronial tissue donation Police Services				
PALLA A 1				
5 Brack proming				
court attendance and delivery of expert evidence in court accident and crime scene evidence analysis.				
accident and crime scene evidence analysis Forensic Chemistry Illicit drug analysis				
imor and analysis				
Clandestine laboratory analysis and remediation				
Physical evidence examination				
Oil spill comparisons				
Organic Chemistry Testing for organic and pesticide contaminants in recycled and				
in the state of th				
drinking water, biological specimens and foods				
Air analysis for volatile organic/compounds				
Algae identification and toxin analysis				
 Food testing to determine safety, chemical composition and 				
compliance				
 Molecular testing to determine the identity and country of origin 				
of foodstuffs				
rganic Chemistry . Testing for metal and inorganic compounds in the environment				
consumer products, biological materials and food				
Environmental monitoring of nutrients				
Microbiology • Food and water microbiology				
Reference microbiology				
Molecular epidemiology				
Reference and research of Leptospirosis				
 Virology Serology diagnostic laboratory Molecular diagnostic laboratory 				
Virus culture				
Research entomology				
Radiation and • radiation assessments for public and environmental health				
Nuclear Science programs				
sontaminated land assessment and radiological baseline				
/) 5110100				
studies				
radiation testing of food, water and soil				
radiation testing of food, water and soil radiation survey meter calibration				
radiation testing of food, water and soil radiation survey meter calibration waste disposal and transport of radioactive substances				
radiation testing of food, water and soil radiation survey meter calibration waste disposal and transport of radioactive substances radiation safety training 				
radiation testing of food, water and soil radiation survey meter calibration waste disposal and transport of radioactive substances radiation safety training compliance testing and certification of radiation sources, 				
radiation testing of food, water and soil radiation survey meter calibration waste disposal and transport of radioactive substances radiation safety training compliance testing and certification of radiation sources, equipment and premises.				
radiation testing of food, water and soil radiation survey meter calibration waste disposal and transport of radioactive substances radiation safety training compliance testing and certification of radiation sources, 				

- obtain, document and interpret medical evidence from victims of crime, alleged offenders and examination of adult sexual assault victims
- provide toxicological advice to the Coroner and courts in relation to suspicious deaths and driving matters



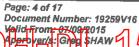
- provide expert evidence and appear in court
- examine the treatment of police detainees
- support the Office of the State Coroner in relation to the investigation of healthcarerelated deaths.

These services are supported by;

- Client Support and Liaison
- Campus Management and Support
- Scientific Support

The corresponding services provided by these units is detailed below;

	Client Support and Liaison (///
Public Health	responsible for receiving, registering, tracking, storing, coding,
Property Point	
	allocating and delivering public health samples to the scientific business units at FSS
Forensic Property	
Point	responsible for receiving, registering, tracking, storing, coding,
	allocating and delivering forensic exhibits to the scientific business
Scientific Services	units at FSS
Liaison Unit	responsible for the effective ligison between FSS and key clients
	such as Queensland Police, and Department of Justice and
	Attorney-General. The unit ensure correct case allocation to the
	Forensic scientists and is responsible for court scheduling etc
Pr 1114	Campus Management and Support
Facilities Management	responsible for the maintenance of campus buildings and
Management	related infrastructure
	responsible for coordinating contractors brought on site to
	complete works
Operational Support Services	responsible for contract management and delivery of cleaning,
Services /	security, grounds, waste and hygiene services.
/ /	The area is also responsible for vehicle fleet management, internal
	sourier service and a centralised warehouse, tracking all incoming
	and outgoing freight for laboratories; as well as the
	commencement of the asset lifecycle process
Tenancy and	responsible for business management and information (finance,
Business Management	billing, fleet vehicle transactions, petty cash, coordinating business
Iviariagement	case process, purchase requisitions) as well as executive and
~	administrative support; providing relief to laboratories for admin
	functions and secretariat functions to executive management
Records	responsible for right to information (RTI) request for records and
Management	supporting the QH Legal Unit. The area is also responsible for
	managing all corporate records information in relation to strategic,
	operational, financial and decision making into FSS funding,
;	revenue and resources. The unit provides advice and training to
	The unit provides advice and training to





	laboratories across the campus to capture, manage, archive and lawfully destroy records in compliance to Public Records Act 2002			
	Scientific Support			
Scientific Skills Development Unit	 Facilitates the development of a learning culture across all business units at FSS. Responsible for leading the design, development, implementation and evaluation of competency based training programs across FSS. Delivers scientific and related training facilitating and coordinating the provision of other training development services facilitates implementation of court training for scientists providing evidence in court 			
Quality and Regulatory Compliance	responsible for managing, maintaining and improving all aspects the FSS quality system including accreditation, certification and any regulatory and legislative requirements relating to these issues. The role provides authoritative, professional advice and assistance to management, supervisors and employees on quality systems issues			
Research and Development	 provides assistance to FSS staff with application and approval documentation monitors and reviews project progress and budget assists with collation and submission of grant applications to facilitates training in research and development areas liaises with Legal and Administrative Law Unit (LALU), universities and other government departments for administration of cross-jurisdictional projects and contracts facilitates Site Specific Assessment for projects requiring ethical approval Facilitates FSS Human Research Ethics Committee 			
Information and Research Services	Provides information and research support to FSS services, including literature searches, electronic journals and databases, epririts (FSS staff publications), access to Australian Standards and lending services			
(() [7			

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Archerfield 4108

Website: www.health.qld.gov.au/qhcss/qhss



Hours of Operation

FSS routinely operates Monday – Friday 0830 - 1630. Some laboratories operate extended hours or have on-call arrangements.

Clients

The client base includes the Department of Health, Queensland Police Service, Office of the State Coroner, Department of Justice and Attorney-General, other government departments and the private sector.

Governance

The <u>FSS Governance Manual (20033)</u> provides an overview of the corporate governance processes of FSS, including organisational and committee structures as well as outlining reporting procedures and requirements. It also profiles the organisations senior management responsibilities.

Business Continuity

Each unit has a Business Continuity plan, which is governed by the FSS Business Continuity Management Framework (27063).

Disaster Management

The FSS Disaster Management Plan (28736) is a functional sub-plan for the Queensland Health Disaster and Emergency Management Arrangements. The plan provides for an all hazards, all agencies, and comprehensive approach to emergency management.

Planning

The FSS Operational Plan is a result of the FSS Service planning process. There is regular monitoring and reporting against the Operational Plan at the FSS Executive, and the plan is reviewed to ensure it reflects changes in the environment.

Quality Policy

The Quality Management System of FSS is governed by the FSS Quality Commitment (33322), and uses processes aligned with HSQ. These processes are regularly reviewed by the FSS Quality Advisor for ongoing relevance.

The FSS Quality Management System supports several accreditations, certifications and approvals as listed in section 7 of this document. FSS management is committed to continual improvement through the implementation and maintenance of the quality management system, and conformance to the relevant standards and guidelines that pertain to the organisation.

In addition to the principles outlined in the FSS Quality Commitment, the FSS Quality Management System will ensure and maintain the quality of all aspects of reference material production, including material quality, characterisation, assignment of property values and material handling storage and transport procedures. The reference material producers within FSS will be familiar with production systems, will produce reference materials that conform to the relevant international standards, and are committed to including the use of inter-laboratory characterisation studies where appropriate, and interacting with the appropriate sectors of the measurement community.

5 Personnel

Confidentiality

FSS staff are governed by the <u>Queensland Public Service Code of Conduct</u>, and section 3 of the Analytical Services Framework <u>14475</u>.



Orientation and Induction

All new staff participate in a site induction on day one of employment, and are given copies of the Campus Operations Manual (28335). All staff are required to complete their area induction and mandatory training within 3 months of employment.

All FSS staff are required to have current mandatory training. Details of mandatory training requirements are found on the SSDU website (under induction).

Payroll and rostering

FSS staff are supported by Campus Management and Support, who facilitate all employee related issues such as pay enquiries and leave applications. All relevant forms and industrial relations manuals can be found at either the <u>HSQ HR</u> page or at the <u>PARIS</u> home page.

All FSS staff are required to complete an electronic timesheet. Refer to QIS doc 25310.

QIS#	Title	Description
<u>25310</u>	Time Sheet management	Describes the preparation of and completion of
	procedure	timesheets for FSS staff

Position Descriptions

Each position within FSS has a documented position description, available from Campus Management and Support. Position Descriptions are used in the recruitment and selection process, and for annual performance and development plans (PDP). Further information about the PDP, and links to relevant documentation can be found at http://gheps.health.gld.gov.au/hr/pdp/performance-dev-planning.htm.

Training and Competency of Staff

The Scientific Skills Development Unit (SSDV) coordinates the training needs and professional development of FSS staff. Competency is assessed via completion of training modules (23277), or statement of competence (23947) and on-going assessment is through annual performance and development plans (PDP). Hard copy records are kept for each individual. PDP and competency is recorded in the Professional Development module of QIS (29249). The Professional Development module of QIS is also used to record qualifications, experience and other professional development activities such as publications and conference attendance (26208).

The FSS Learning and Development Guidelines (23651) describes the framework for FSS staff learning and development. The effectiveness of training is evaluated as outlined in section 4 of this document, and also through the following:

- Feedback forms
- Debrief sessions for trainers
- Morithly trainers meetings
- Training delivery plans
- Comments and suggestions for improvements on training modules
- Opportunity to provide feedback in training module
- FSS Training email
- Evaluations performed by SSDU training coordinator

Continuing Education and Professional Development

The continuing education and professional development of FSS staff is supported by the SSDU and the Information and Research Service.

The SSDU facilitates course and conference attendance. Details are available via their website.

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Services offered by the Information and Research Service can be accessed through their website.

Other resources such as allowable internet sites are available through the <u>HSQ training and development site</u>.

Procedures specific for FSS are:

QIS#	Title	Description
10675	FSS - Application for Professional Activity Leave	Application for Professional Activity Leave to attend Conferences, Seminars, Meetings, Training Courses and Project Related Travel where financial outlays by FSS and/or leave of absence for staff are involved
33148	Performance Development Plan	Template used by PSS

Safety

The occupational health and safety of FSS staff is facilitated by the HSQ Occupational Health and Safety Unit.

QIS#	Title	Description
<u>10738</u>	Personal Protective Equipment Policy and Procedure	Defines general rules for the use of Personal
	Policy and Procedure	Protective Equipment (PPE) in FSS. Individual workgroups may have additional or special
		requirements. These are to be documented in
		specialised SOPs or in relevant test methods
28958	Environmental and Waste	This document outlines site requirements for
	Management Plan	staff, tenants and contractors relating to waster
		nagement nagement

6 Administration

The following procedures are related to administrative activities performed within FSS;

QIS#	Title	Description
19981	Business Case Management	Procedure for preparation and approval of
	at FSS Standard Operating	business cases, change initiatives and project
	Procedure (SQP)	proposals at Forensic and Scientific Services
28714	FSS Correspondence	Assists authors in the development of
	Checklist	executive documents and correspondence.
10637	Procedure for Booking an	Describes the process to book a conference
	Conference Room	room at FSS.
<u>10646</u>	FSS Vehicle Management	Details procedure for borrowing Queensland
		Government vehicles from FSS
<u>30655</u>	Completion of Vehicle Log	Details process for Completion of Vehicle Log
	Sheets	Sheets
30702	Use of FSS pool vehicles by	Describes process for use of FSS pool
	laboratory staff carrying out	vehicles by laboratory staff carrying out
	sampling in the field	sampling in the field
<u>25361</u>	Procedure for distribution of	Describes the procedure for sending FSS
	broadcast e-mail	broadcast emails
<u>25739</u>	Cab Charge/E-Tickets	Describes the process in the handling of taxi /
		cab charge E-Tickets



20089	FSS Infrequent Testing Guideline	Provides a guideline to FSS laboratories that perform infrequent tests, to ensure the competency of the analyst performing the test, and the currency of the methodology used.
30767	FSS standards for Analytical Spreadsheets	Describes the minimum standards to be followed by FSS for analytical spreadsheets

Test methods used by the laboratory are detailed in separate test methods managed in the Quality Information System (QIS). Acceptance criteria for test results are contained in these test methods.

Internal QC

Specific methods contain requirements for internal quality control and rules for their acceptance/rejection.

Reports

Test reports issued by FSS are NATA endorsed where possible. Some labs have decided to only issue endorsed reports when requested by the client, as the lab is unwilling to accept the risk of reports being recalled if found not to comply with NATA requirements.

FSS has received Crown Law advice that electronically signed analyst certificates are admissible as evidence in court proceedings.

Electronic signatures (e-signatures) are stored in Auslah and are linked to the individuals unique login. Use of Auslah for e-signatures ensures that they are kept secure and prevents unauthorised use, as the signature appearing on the certificate is the login of the person validating the result (F6). Auslah also maintains an audit trail for all activities performed in the system. E-signatures shall not be used for offline reports.

Reports issued as part of the ENCT proficiency testing scheme are the property of the participant, and may be used as required by the participant.

The following are procedures specific to reports issued by FSS laboratories:

QIS#	Title	Description
	FSS - Laboratory Report	
10023	Format on Contract	Defines requirements for the format and
	Format and Content	content of laboratory reports issued by FSS
<u>14076</u>	Test Records	Ensures that laboratory records contain the
		appropriate information for all tests and related
	\sim	activities and that they are consistently
	\(\sigma\)	handled in a manner that retains their integrity
\ \ \/	(/)	without loss and/or damage
26993	Procedure for authorising staff	
*****		Outlines the procedure for authorising staff to
1	to release results for NATA	release results for NATA accredited tests
	accredited tests	
18034	FSS Court Testimony and	Describes the process associated with court
	Attendance Requirements	testimony
29024	Use of offline Forensic	Describes the use of offline templates for
	reporting templates	statement of witness and certificate of analysis
	reperting templated	
		for laboratories reporting in the forensic
00405	11 6 60 5 11 11	division of AUSLAB
<u>29125</u>	Use of offline Public Health	Contains instructions on the use of offline
	reporting templates	reporting templates in FSS Public Health
<u> </u>		laboratories



Proficiency Testing

Team leaders and Managing Scientists are responsible for ensuring that proficiency testing (PT) samples and results are handled in accordance with NATA accreditation requirements (eg mimic normal testing processes, are reviewed, and corrective action taken where appropriate). They are also responsible for maintaining records of participation for an appropriate period.

The FSS Quality Office is responsible for organising participation in certain PT programs, raising OQI's where required, and forwarding PT results to relevant areas.

PT with a requirement to have one point of contact for enrolment (eg NIFS, PTA-Chemical) are coordinated through the FSS Quality Office. Programs where only one laboratory is participating are organised directly by the lab. Samples and reports that are received by the Quality Office are forwarded to the appropriate team leader or their delegate. Selection of PT providers is informed by details available on the NATA website

Laboratories are encouraged to participate in accredited schemes and as broad a range of proficiency testing activities as practicable and available. Labs must participate in proficiency testing in accordance with the frequency stipulated by NATA.

8 Finance

Finance procedures specific to FSS are outlined below

QIS#	Title	Description
		Outlines the procedure to be followed to
	Line Managers with Financial	ensure relief for line manager has appropriate
	Delegation /	financial delegation

9 Records

Records at FSS are created, retained and disposed of according to the Records Management Policy, Standards and Guidelines.

Procedures specific for FSS are:

QIS#	Title	Description
10625	FSS - Records creation,	Provides details of procedures to be followed
	retention and disposal	to manage records at FSS
<u>28965</u>	Key to FSS Onsite Records	Provides information to FSS employees on
	V	where the onsite storage facilities are located,
		who has control and ownership of the facility and how to access records
23201	Destruction of Ephemeral	Ensures that all FSS records deemed ready
	(Information Only) Records	for destruction are destroyed in a controlled,
	~	secure environment in compliance with the QH Records Management Policy
<u>14080</u>	Records Management Data	Describes procedure to enter data consistently
	Entry Standards	and accurately into QH recordkeeping
		application system
<u>19781</u>	RecFind Searching Procedures	Provides RecFind (or RecQuery) users with
		procedures to successfully search for data
		within the systems
<u>27115</u>	Corporate File Management	Describes the process for Corporate File



		Management including, file creation, applying disposal timeframes, managing physical files and recording movement of files
27131	Destruction of QH Records	Describes the process for approving the
	(FSS)	destruction or extension of records retention

10 Purchasing

Purchasing by FSS is governed by the Department of Health policies. Procedures specific to purchasing at FSS are detailed below;

QIS#	Title	Description
10628	Procedure for engaging and assessing new vendors	Describes how to assess and record the suitability of suppliers and vendors of consumables, capital items and services
10622	Verification of Consumables within the Work Group	Describes procedures for ascertaining whether consumables are fit for purpose. Labelling and record keeping are included.
26627	How to Access the FSS Procurement Advisory Service	Outlines the process for contacting the Forensic and Scientific Services (FSS) Procurement Advisory Service and the procedure for completing a "Request for Quotation" form.
<u>32164</u>	Minor Works Request	Template to be used where a work area requires minor works to be completed
32129	Scientific Equipment Service Maintenance Agreement Request	Template to be used to request instigation of a service maintenance agreement for scientific equipment
32422	Building Plant and Equipment Maintenance Agreement Request	Template for funding approval, to be used where a maintenance agreement is required for building related plant and equipment
27670	FSS Disposal Of Waste Chemicals Procedure	Process for disposal of waste chemicals at FSS

11 Equipment

Equipment Inventory

An Equipment Inventory is available using the Calibration module of the Quality Information System (QIS).

Calibration and Maintenance of Equipment

Calibration and checking intervals are detailed by NATA in the Reference and General Equipment tables. These intervals are reflected in the Calibration module of QIS. Calibration and checking of equipment is supported by other quality assurance activities conducted by the laboratory such as performance of internal quality control checks, routine use of spikes, routine use of reference material, and participation in external quality assurance programs. These additional checks are an immediate indication of the validity of the test results, and an indicator of the correct functioning of the equipment used.

NATA encourages the development of an equipment surveillance program, which moves the emphasis from a reliance on the demonstration of equipment conformance, to a greater contribution from more frequent checks against reference materials, and checking of critical features. In the absence of such a program, the NATA requirements are to be followed.



Due to the legal nature of a significant amount of the work performed by FSS, the Senior Director has decided that, unless expressly specified, equipment calibration intervals will follow the requirements of NATA, and no equipment surveillance programs will be developed.

Critical equipment is defined at FSS as 'pieces of equipment that directly affect the reported test result, and whose failure would not be detected by test controls'.

Equipment is maintained in accordance with the HSQ guidelines contained in QiS <u>26325</u>. Calibration procedures that apply to equipment commonly in use at FSS are detailed below;

QIS#	Title	Description
10666	HSQ - Balance Verification	Describes the on use, monthly and six-monthly
	and Assessment	verification checks of electronic balances.
10668	Procedure for acceptance	Describes the prodedure for conducting
	checking and calibration of	acceptance checks and full calibrations of
	volumetric glassware	items of glassware used in situations where
		volumetric errors could)contribute significantly
40000		to analytical errors
10669	Verification Of Environmentally	Describes the procedure for checking
40070	Controlled Enclosures (ECE)	environmentally controlled enclosures.
10670	Procedure for Verification of	The purpose of this procedure is to ensure that
	Thermometers	all liquid-in-glass and digital thermometers
		within FSS requiring verification are checked in
		accordance with ISO 17025, NATA and any
10671	HSQ - The Calibration Of	legislative requirements Descripes the calibration procedures for
10011	Piston Operated Volumetric	piston-operated volumetric apparatus
	Apparatus (POVA)	platon-operated volumetric apparatus
<u>10672</u>	HSQ - The Verification of	The purpose of this procedure is to ensure that
	Timing Devices //)	stopwatches and other timing devices which
		require precise verification are checked in
		accordance with ISO 17025 and NATA
00004		requirements
26931	Accessing the maintenance	Outlines the type of documents stored in the
	records management system	maintenance records management system
		(RMS) and how to access the documents
<u>24805</u>	FSS Disposal of Equipment	Describes the steps to take in order to dispose
	Procedure	of FSS equipment which may be broken,
	(A) *	superseded or unwanted.

12 Research

Research conducted at FSS is coordinated through the <u>Research and Development Office</u>. FSS supports research through the following;

Q!S #	Title	Description	
10642	FSS Animal Ethics Committee Terms of Reference and Operational Procedures	Describes the terms of reference and operational procedures for the FSS Animal Ethics Committee	
10643	FSS - Institutional Biosafety Committee	Defines the functions and terms of reference of the Forensic and Scientific Services Institutional	



		Biosafety Committee (FSS-IBC), which deals with genetic manipulation issues.
10664	FSS - Human Ethics Committee Functions and Terms of Reference	Describes the composition and operation of the Human Ethics Committee
14079	FSS Medical Advisory Committee – Functions and Terms of Reference	Describes the composition and operation of the Committee, whose principal role is to put into place procedures to be followed in the event of an accident, or exposure of staff to bacterial, viral or rickettsial agents, in the FSS PC3 or PC4 suites
10644	Importation of Exotic Viruses	Describes the procedures necessary of import of level 4 viruses

13 Quality Management System

The Quality Management System is coordinated through the QIS (Quality Information System) database. Flowcharts detailing how to use QIS are contained in the Quality Information System user manuals (26207 – 26214, 2024).

Procedures specific to the functioning of the Quality system at FSS are below;

		7//
QIS#	Title	Description
<u>26370</u>	Procedure for staff changes in QIS2	Describes the processes to be
		followed by FSS staff and the QIS admin team to manage staff changes in QIS
26369	Procedure for adding new documents and QIS Administrators	Provides FSS staff with instructions on how to get a new document added to QIS, and provides them with information on their QIS2 administrators

Quality Indicators /

Current quality indicators for FSS are:

- 1. critical documents current
- 2. critical QQI's actioned in 30 days. Critical QQI's are external complaints, safety issues and clinical incidents
- 3. external agency non-conformances actioned in 30 days

Document Control

The Procedure for Document Management 10003 outlines the control, development, publication, review/amendment and withdrawal of all documents. Documents are classified according to the hierarchy as detailed in the HSQ Document Hierarchy, Document Types and Document Authorisation Policy 24756. The recommended review period for FSS documents is 24 months.

QIS allows for access to documents to be limited through the private viewers functionality. The use of this functionality to protect the content of documents is not encouraged by HSQ, however it is recognised that FSS deals with issues of an extremely sensitive nature, and the protection of documents is necessary in some cases. Authorisation for protection of



documents lies with the relevant Managing Scientist or Unit Manager. Guidelines for FSS Units for the types of documents that should be protected are detailed below;

FSS Unit	Guideline	
Public and Environmental Health	Security Sensitive or commercial-in-confidence	
Coronial Services	Security Sensitive	
Police Services	Security Sensitive	
Campus Management and Support	No need for password protected documents identified	
Scientific Support	No need for password protected documents identified	
Client Support and Liaison	No need for password protected documents identified	

Corrective/Preventive Action and Complaints

The procedure for Management of Opportunities for Quality Improvement (OQI) 13965 describes the creation and resolution of OQI's as well as the timeframes and escalation process surrounding them.

Internal Audit

The Internal Audit Procedure 10636 describes the procedure followed to perform internal audits within Health Support Queensland (HSQ). These audits aim to identify critical processes of the organisation, associated risks and the effectiveness of controls. They also seek to identify improvements and whether all requirements of relevant standards are met. This procedure is supplemented by numerous audit checklists available on QIS. It is intended that these checklists are used as a guide for auditors to develop their own process audit checklist.

QIS#	Title
19130	Audit checklist - general
20088	ISO 9001 checklist
20030	ISO/IEC 17025 checklist
20032	AS/ISO 15189 chesklist
20026	ISO Guide 34 (Reference Material Producers) audit checklist
20029	ISO 17043 (Proficiency Testing) audit checklist

An audit schedule can be obtained from the Quality Information System (QIS). Internal Auditor Training is conducted at FSS by the Principal Quality Advisor (see FSS Quality Page for course details). Training is conducted in accordance with the HSQ Internal Auditor Training Module (24740). A list of trained auditors can be obtained from the HSQ staff directory, with internal auditors being identified through the symbol ...

Management Review

The Management Review Procedure 10010 describes the process by which management formally conducts periodic, independent reviews of the management system.

Management review is performed on at least an annual basis by the FSS Executive. The meeting follows an agenda, and outcomes of the meeting are communicated to all FSS staff via Managers and Managing Scientists. Monthly status reports (including



management review) are provided by the FSS Quality Advisor to FSS Executive, which can provide the report to their respective work units.

The Quality Community of Practice, organised by the FSS Quality Advisor, regularly reviews overdue QIS events in each area.

Management review is also part of regular team meetings, which are conducted in accordance with the document Requirements for Team Meetings 17577

Client Feedback

FSS strives to actively seek feedback, both positive and negative, from its clients. This is facilitated through the Scientific Services Liaison Unit (28520). Feedback is sought both formally and informally, electronically (through the use of email and phone meetings), and face to face (via meetings, conferences, working groups etc). Minutes, issues logs, and OQI's are used to record client feedback.

14 Amendment History

Version	Date	Updated by	Amendments
0-13	Various	Various	See archived versions
14	Feb 2015	H Gregg	Clarification of ISO 9001 certification in Section 7
15	Apr 2015	H Gregg	Statement on use of PT reports added to 'reports' section
16	Sept 2015	H Gregg	Updated OHS sections to reflect organisational restructure. Updated PT participation interval in
			section 7 Updated to new template Removed table of contents





EndPoint Analysis Report

Experiment Information

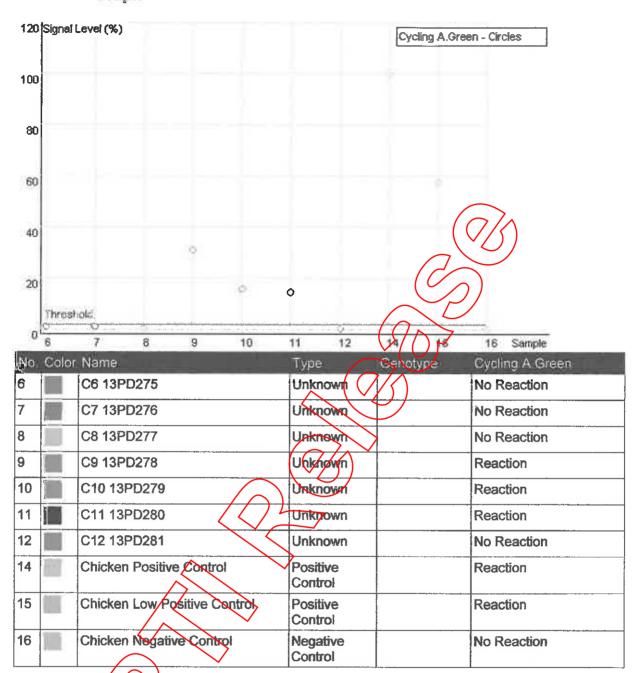
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Run Finish	1/05/2014 7:11:01 PM	
Operator	Glenngy	
Notes		
Run On Software Version	Rotor-Gene Q Software 2.0.3.2	
Run Signature	The Run Signature is valid.	
Gain Green	6.	
Machine Serial No.	0410161	(0)

End Point Information

Imported Analysis Settings	
Threshold	% 2

Cycle	Cycle Point	
Hold	Hold @ 95°C, 9min/0s	
Cycling (45 repeats)	Step 1: Hold @ 94°C, 30s	
	Step 2; And @ 60°C, 39's	
	Step 3 Hold @ 72°C, 30s, acquiring to Cycling A([Green][1][1])	
Melt	Ramp from 76°C to 89°C	
	Hold for 90s on the 1st step	
Hold for 5s on next steps, Melt A([Green][1][1])		

EndPoint Graph



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EndPoint Analysis Report

Experiment Information

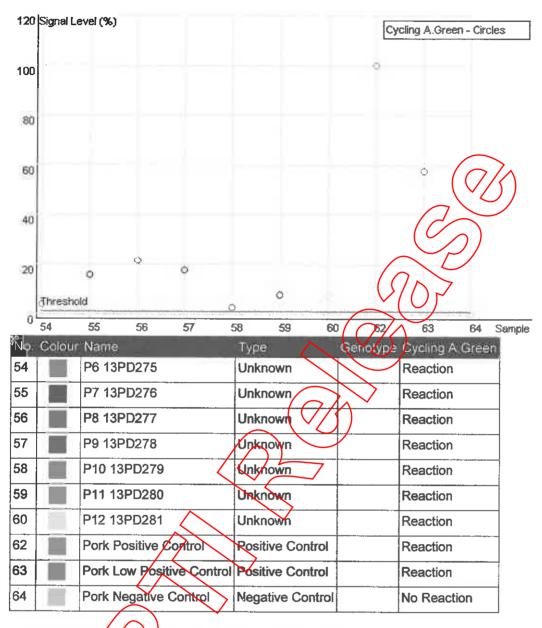
Run Name	Glenn's SIM Meat Assay 72 W	ell 2013-05-30 (1) 2014-05-01 (1)
Run Start	5/1/2014 4:59:31 PM	
Run Finish	5/1/2014 7:11:01 PM	$(\sqrt{3})$
Operator	Glenngy	
Notes		
Run On Software Version	Rotor-Gene 2.0.3.2	
Run Signature	The Run Signature is valid.	
Gain Green	6.	V/07

End Point Information

Imported Analysis Settings	
Threshold	1%

Cycle	Cycle Point
Hold @ 95°c, 9 min 0 secs	
Cycling (45 repeats)	Step 1 @ 94°c, hold 30 secs
	Step 2 @ 60°c, hold 30 secs
	Step 3 @ 72°c, hold 30 secs, acquiring to Cycling A([Green][1][1])
Melt (76-89°c), hold sees on the 1st step, hold 5 secs on	
next steps, Melt A([Green][1][1])	

EndPoint Graph



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EndPoint Analysis Report

Experiment Information

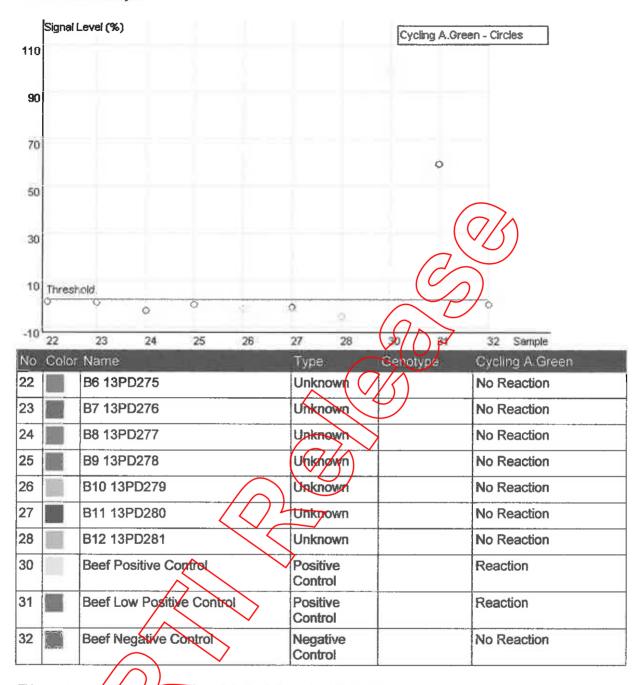
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Run Start	1/05/2014 4:59:31 PM	
Run Finish	1/05/2014 7:11:01 PM	The second secon
Operator	Glenngy	
Notes		
Run On Software Version	Rotor-Gene Q Software 2.0.3.2	
Run Signature	The Run Signature is valid.	
Gain Green	6.	
Machine Serial No.	0410161	O_{N}

End Point Information

Imported Analysis Settings	
Threshold	% 2

Cycle	Cycle Point
Hold	Hold @ 95°C, 9min 0s
Cycling (45 repeats)	Step 1: Hold @ 94°C, 30s
	Step 2: Hold @ 60°C, 30's
	Step 3 Hold @ 72°C, 30s, acquiring to Cycling A([Green][1][1])
Melt	Ramp from 76°C to 89°C
	Hold for 90s on the 1st step
	Hold for 5s on next steps, Melt A([Green][1][1])

EndPoint Graph



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EndPoint Analysis Report

Experiment Information

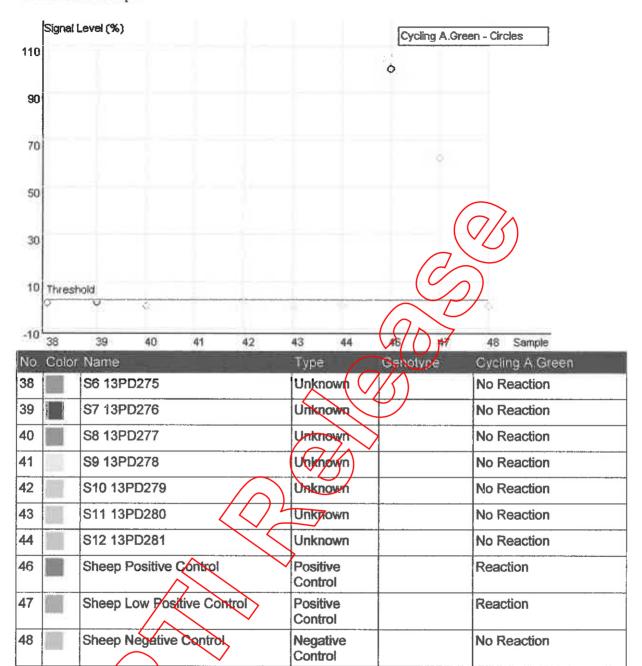
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Run Finish	1/05/2014 7:11:01 PM
Operator	Glenngy
Notes	$(\checkmark/)$
Run On Software Version	Rotor-Gene Q Software 2.0.3.2
Run Signature	The Run Signature is valid.
Gain Green	6.
Machine Serial No.	0410161

End Point Information

Imported Analysis Settings		7
Threshold	% 3	,

Cycle	Cycle Point
Hold	Hold @ 95°C, 9min 0s
Cycling (45 repeats)	Step 1: Hold @ 94°C, 30s
	Step 2; All @ 60°C, 39's
	Step 3: Hold @ 72°C, 30s, acquiring to Cycling A([Green][1][1])
Melt	Ramp from 76°C to 89°C
	Hold for 9% on the 1st step
	Hold for 5s on next steps,Melt A([Green][1][1])

EndPoint Graph



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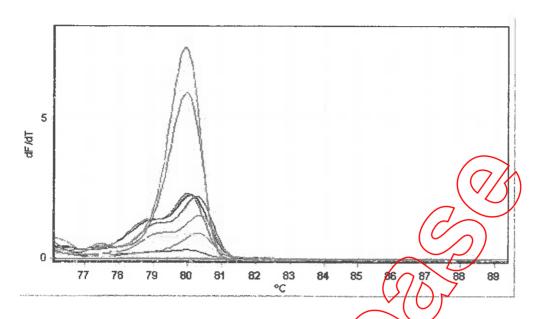


Melt Report

Experiment Information

Run Name	Glenn's SIM Meat Assay 72 We	ell 2013-05-30 (1) 2014-05-01 (1)			
Run Start	5/1/2014 4:59:31 PM				
Run Finish	5/1/2014 7:11:01 PM				
Operator	Glenngy				
Notes					
Run On Software Version	Rotor-Gene 2.0.3.2				
Run Signature	The Run Signature is valid.				
Gain Green	6.	V/0r			
Melt Information		7/5)			
Digital Filter	Light				
Imported Analysis Settings					
Sample Page	Page 1 ((// ^				
Temp. Threshold	0°c				
Threshold	0.				
Messages					
Profile					
Cycle		Cycle Point			
Hold @ 95°c, 9 min 0 secs					
Cycling (45 repeats)		Step 1 @ 94°c, hold 30 secs			
		Step 2 @ 60°c, hold 30 secs			
		Step 3 @ 72°c, hold 30 secs, acquiring to Cycling A([Green][1][1])			
Melt (76-89°c) , hold secs next steps, Melt A([Green]	on the 1st step, hold 5 secs on [1][1])				

Melt data for Melt A. Green



ļγo	Colour	Name	Genotype	Peak 1	(Peg/Z	Peak 3	Peak 4	Peak 5	Peak 6	Реак 7	Peak 8
54		P6 13PD275		77.38	79.94						
55		P7 13PD276		80.10	82.56	83.54	84.46	85.74	86.66		
56		P8 13PD277		76,90	78.96	80.00					
57		P9 13PD278		76.30	80.26	84.06	85.20	86.00	86.74	87.66	88.80
58		P10 13PD279	$\bigcap L$	77.54	80.30						
59		P11 13PD280		76.90	80.36						
60		P12 13PD281		77.16	79.10	80.00					
62		Pork Positive Control		77.46	79.94						
63		Pork Low Positive Control		79.96							
64		Pork Negative Control		77.44	78.24	79.06	79.84	81.30			

Biri Name Terripetaty/e Sample No. Sample Name Peak

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Melt Report

Experiment Information

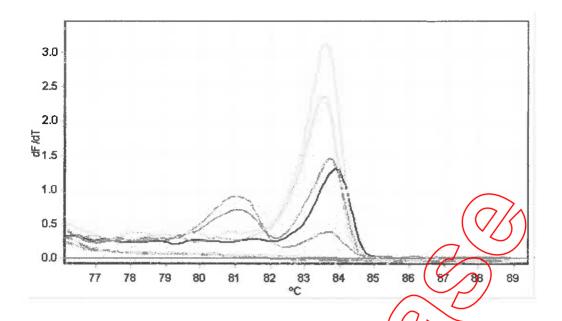
Run Name	Glenn's SIM Meat Assay 72 Well	2013-05-30 (1) 2014-05-01 (1)
Run Start	1/05/2014 4:59:31 PM	
Run Finish	1/05/2014 7:11:01 PM	
Operator	Glenngy	$\langle \alpha \rangle_{\Lambda}$
Notes		
Run On Software Version	Rotor-Gene Q Software 2.0.3.2	
Run Signature	The Run Signature is valid.	
Gain Green	6.	
Machine Serial No.	0410161	(0)

Melt Information

Digital Filter	Light
Imported Analysis Settings	
Sample Page	Page 1
Temp. Threshold	0°C
Threshold	0.

-Cycle	Cycle Point
Hold	Hold @ 95°C, 9min 0s
Cycling (45 repeats)	Step 1: Hold @ 947C, 30s
	Step 2: Hold @ 60°C, 30s
	Step 3: Hold @ 72°C, 30s, acquiring to Cycling A([Green][1][1])
Melt /	Ramp from 76°C to 89°C
	Hold for 90s on the 1st step
	Hold for 5s on next steps,Melt A([Green][1][1])

Melt data for Melt A.Green



No.	Color	Name	Genotype	Flesh	Peak 2	Peak 3	Peak 4
6	0 0	C6 13PD275	(0)/	79 .16	79.90		
7		C7 13PD276		79.70	81.64		
8		C8 13PD277		77.80	79.16	80.04	80.90
9		C9 13PD278		77.60	78.50	81.06	83.70
10		C10 13PD279	<i>\)</i>	76.40	77.36	78.90	81.00
11		C11 13PD280		76.24	77.30	78.20	78.76
12		C12 13PD281		78.54	79.70	81.84	
14		Chicken Positive Control		78.20	78.86	79.80	80.90
15		Chicken Low Positive Control		76.90	78.50	79.30	80.54
16		Chicken Negative Control		77.44	78.50	79.40	80.26

Bin Name Temperature Sample No. Sample Name Peak

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Melt Report

Experiment Information

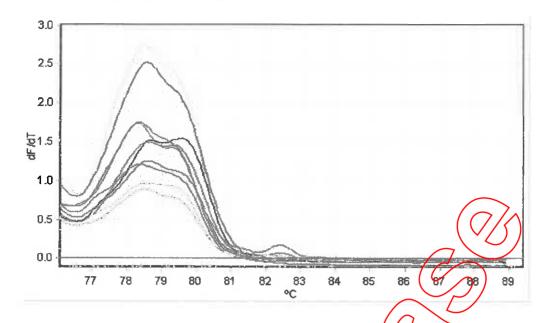
Run Name	Glenn's SIM Meat Assay 72 Well 2013-05-30 (1) 2014-05-01 (1)		
Run Start	1/05/2014 4:59:31 PM		
Run Finish	1/05/2014 7:11:01 PM		
Operator	Glenngy		
Notes			
Run On Software Version	Rotor-Gene Q Software 2.0.3.2		
Run Signature	The Run Signature is valid.		
Gain Green	6.		
Machine Serial No.	0410161		

Melt Information

Digital Filter	Light
Imported Analysis Settings	
Sample Page	Page 1
Temp. Threshold	0°C
Threshold	0.

-Cycle	Cycle Point
Hold	Hold @ 95°C, 9min 0s
Cycling (45 repeats)	Step 1: Hold @ 94°C, 30s
	Step 2: Hold @ 60°C, 30s
	Step 3: Hold @ 72°C, 30s, acquiring to Cycling A([Green][1][1])
Melt /	Ramp from 76°C to 89°C
	Hold for 90s on the 1st step
	Hold for 5s on next steps,Melt A([Green][1][1])

Melt data for Melt A.Green



No	Color	Name	Genotype	Feak	Peak 2	Peak 3	Peak 4
22		B6 13PD275		78.30	79.26	82.40	83.54
23		B7 13PD276		78.60			
24		B8 13PD277		78.36			
25		B9 13PD278		78.60	79.34	82.44	
26		B10 13PD279		78.54	82.50		
27		B11 13PD280		78.70	79.56		
28		B12 13PD281		78.50	82.04		
30		Beef Positive Control		78.50			
31		Beef Low Positive Control		78.56			
32		Beef Negative Control		78.34	82.54	83.14	

Bin Name Temperature Sample No Sample Name Peak

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Melt Report

Experiment Information

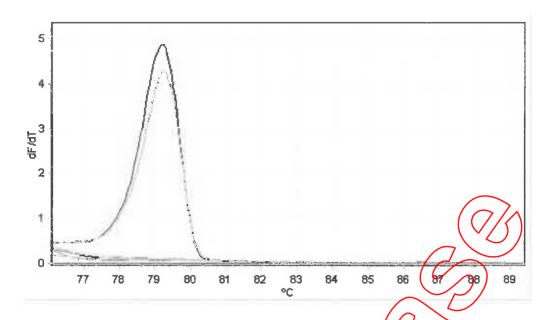
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Run Start	1/05/2014 4:59:31 PM		
Run Finish	1/05/2014 7:11:01 PM		
Operator	Glenngy	(Ω)	
Notes			
Run On Software Version	Rotor-Gene Q Software 2.0.3.2		
Run Signature	The Run Signature is valid.		
Gain Green	6.		
Machine Serial No.	0410161	O_N	

Melt Information

Digital Filter	Light
Imported Analysis Settings	
Sample Page	Page 1
Temp. Threshold	0°C
Threshold	0.

-Cycle	Cycle Point			
Hold	Hold @ 95°C, 9min 0s			
Cycling (45 repeats)	Step 1: Hold @ 94°C, 30s			
	Step 2: Hold @ 60°C, 30s			
	Step 3: Hold @ 72°C, 30s, acquiring to Cycling A([Green][1][1])			
Melt	Ramp from 76°C to 89°C			
	Held for 90s on the 1st step			
	Hold for 5s on next steps,Melt A([Green][1][1])			

Melt data for Melt A.Green



No	Color	Name	Genotype	I Eak	Peak 2	Peak 3	Peak 4
38		S6 13PD275		77.70	78.60	79.40	80.34
39		S7 13PD276		78.60	79.54	80.54	81.94
40		S8 13PD277		77.20	78.60	79.70	82.50
41		S9 13PD278		78.66	79.60		
42		S10 13PD279		78.60	79.60	80.24	
43		S11 13PD280		78.96	81.16		
44		S12 13PD281		78.24	79.30		
46		Sheep Positive Control		79.20	82.40	83.10	84.04
47		Sheep Low Positive Control		76.46	79.24	83.24	84.06
48		Sheep Negative Control		77.30	78.46	79.54	81.90

Bin Name Temperature Sample No. Sample Name Peak

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Quantitation Report

Experiment Information

Run Name	Glenn's SIM Meat Assay 72 Well 2013-05-30 (1) 2014-05-01 (1)		
Run Start	5/1/2014 4:59:31 PM		
Run Finish	5/1/2014 7:11:01 PM	$(\sqrt{/})$	
Operator	Glenngy		
Notes			
Run On Software Version	Rotor-Gene 2.0.3.2		
Run Signature	The Run Signature is valid.		
Gain Green	6.		

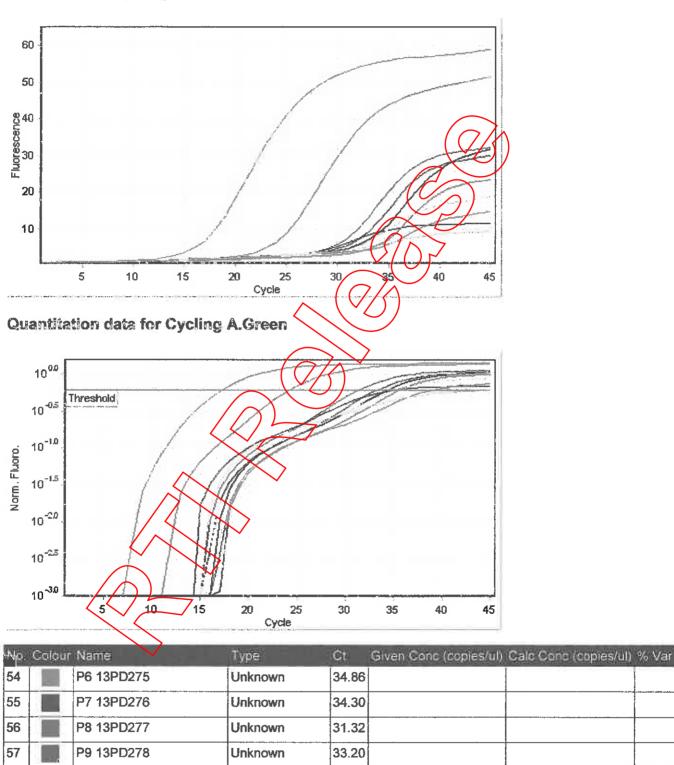
Quantitation Information

Threshold	0.60635
Left Threshold	1.000
Standard Curve Imported	No O
Standard Curve (1)	N/A
Standard Curve (2)	N/A
Start normalising from cycle	1 ()
Noise Slope Correction	No
No Template Control Threshold	0%
Reaction Efficiency Threshold	Disabled
Normalisation Method	Dynamic Tube Normalisation
Digital Filter	Light
Sample Page	Page 1
Imported Analysis Settings	

Cycle	Cycle Point
Hold @ 95°c, 9 min 0 secs	
Cycling (45 repeats)	Step 1 @ 94°c, hold 30 secs
	Step 2 @ 60°c, hold 30 secs
	Step 3 @ 72°c, hold 30 secs, acquiring to Cycling A([Green][1][1])
Melt (76-89°c), hold secs on the 1st step, hold 5 secs on	

next steps, Melt A([Green][1][1])

Raw Data For Cycling A.Green



No.	Colour	Name	Туре	€t	Given Conc (copies/ul)	Calc Conc (copies/ul)	% Var
58	900	P10 13PD279	Unknown	38.74			
59		P11 13PD280	Unknown	35.52			
60		P12 13PD281	Unknown	34.93			
62		Pork Positive Control	Positive Control	17.05			
63		Pork Low Positive Control	Positive Control	24.12			
64		Pork Negative Control	Negative Control	44.31			,

Legend:

NEG (NTC) - Sample cancelled due to NTC Threshold.

NEG (R. Eff) - Sample cancelled as efficiency less than reaction efficiency threshold.

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Quantitation Report

Experiment Information

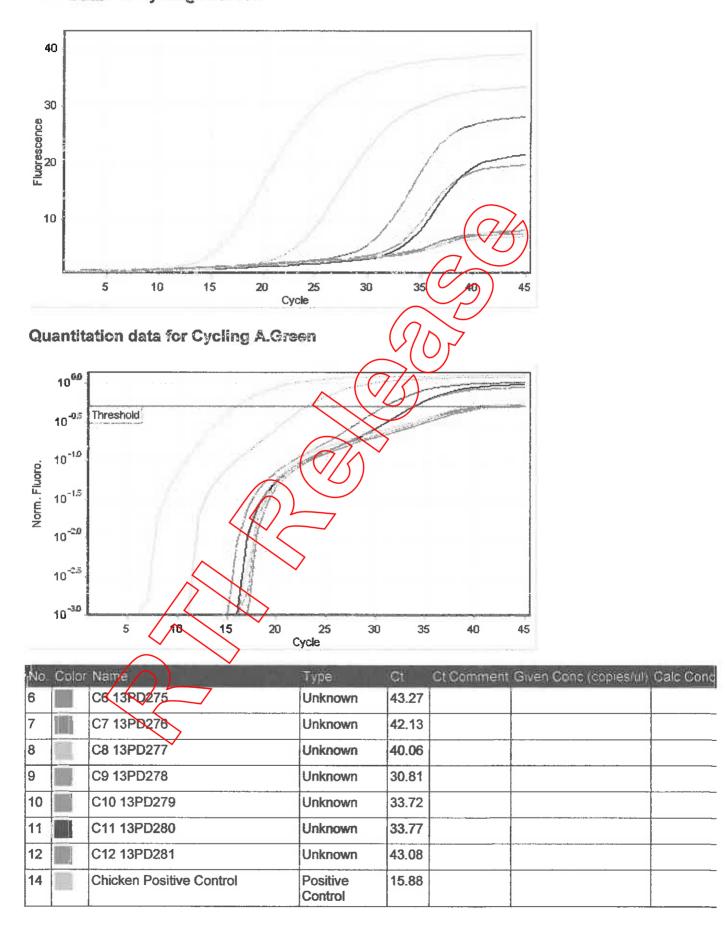
Run Name	Glenn's SIM Meat Assay 72 Well	2013-05-30 (1) 2014-05-01 (1)
Run Start	1/05/2014 4:59:31 PM	
Run Finish	1/05/2014 7:11:01 PM	
Operator	Glenngy	
Notes		
Run On Software Version	Rotor-Gene Q Software 2.0.3.2	
Run Signature	The Run Signature is valid.	
Gain Green	6.	
Machine Serial No.	0410161	100

Quantitation Information

Threshold	0.50582
Left Threshold	1.000
Standard Curve Imported	No
Standard Curve (1)	N/A
Standard Curve (2)	N/A
Start normalising from cycle	1//
Noise Slope Correction	No
No Template Control Threshold	% 0
Reaction Efficiency Threshold	Disabled
Normalisation Method //	Dynamic Tube Normalisation
Digital Filter	Light
Sample Page	Page 1
Imported Analysis Settings	

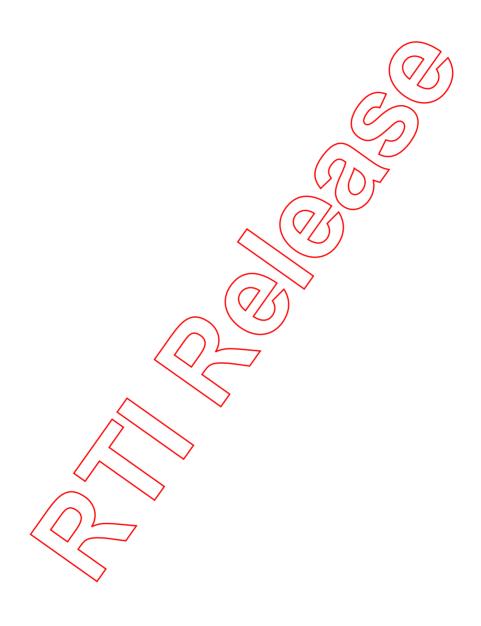
Cycle	Cycle Point
Hold	Hold @ 95°C, 9min 0s
Cycling (45 repeats)	Step 1: Hold @ 94°C, 30s
	Step 2: Hold @ 60°C, 30s
	Step 3: Hold @ 72°C, 30s, acquiring to Cycling A([Green][1][1])
Melt	Ramp from 76°C to 89°C
	Hold for 90s on the 1st step
	Hold for 5s on next steps,Melt A([Green][1][1])

Raw Data For Cycling A.Green



No.	Color	Name	Type	Ct	Ct Comment	Given Conc (copies/ul)	Calc Cond
15		Chicken Low Positive Control	Positive Control	23.06			
16		Chicken Negative Control	Negative Control	44.99			

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Quantitation Report

Experiment Information

Run Name	Glenn's SIM Meat Assay 72 Well 2013-05-30 (1) 2014-05-01 (1)
Run Start	1/05/2014 4:59:31 PM
Run Finish	1/05/2014 7:11:01 PM
Operator	Glenngy
Notes	
Run On Software Version	Rotor-Gene Q Software 2.0.3.2
Run Signature	The Run Signature is valid.
Gain Green	6.
Machine Serial No.	0410161

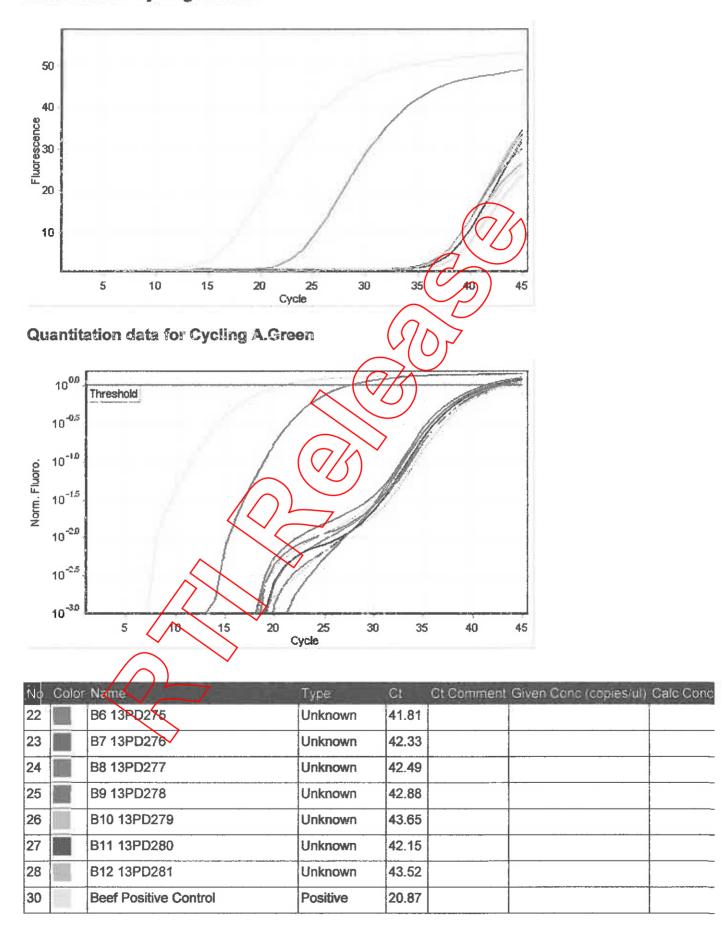
Quantitation Information

1.02205
1.000
No
N/A (7/A)
N/A
1 //)
No
% 0
Disabled
Qynamic Tube Normalisation
Light
Page 1

Profile

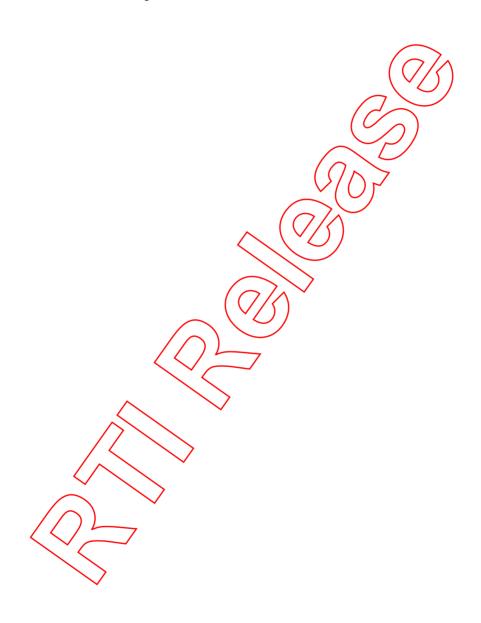
Cycle	Cycle Point
Hold	Hold @ 95°C, 9min 0s
Cycling (45 repeats)	Step 1: Hold @ 94°C, 30s
	Step 2: Hold @ 60°C, 30s
	Step 3: Hold @ 72°C, 30s, acquiring to Cycling A([Green][1][1])
Melt	Ramp from 76°C to 89°C
	Hold for 90s on the 1st step
	Hold for 5s on next steps,Melt A([Green][1][1])

Raw Data For Cycling A.Green



No.	Color	Name	Туре	Ct	Ct Comment	Given Conc (copies/ul)	Calc Conc
			Control				
31		Beef Low Positive Control	Positive Control	27.42			_
32		Beef Negative Control	Negative Control	41.16			

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Quantitation Report

Experiment Information

Run Name	Glenn's SIM Meat Assay 72 Well 2	013-05-30 (1) 2014-05-01 (1)
Run Start	1/05/2014 4:59:31 PM	
Run Finish	1/05/2014 7:11:01 PM	
Operator	Glenngy	
Notes		
Run On Software Version	Rotor-Gene Q Software 2.0.3.2	
Run Signature	The Run Signature is valid.	
Gain Green	6.	
Machine Serial No.	0410161	(0)

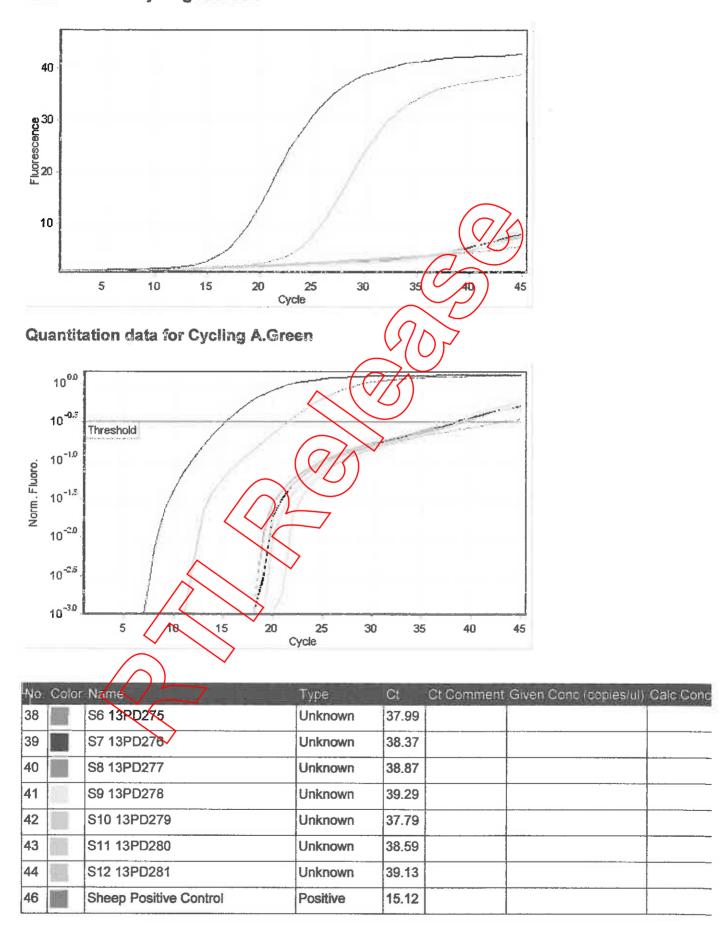
Quantitation Information

0.31543
1.000
No
N/A
N/A
1//
No
% 0
Disabled
Dynamic Tube Normalisation
Light
Page 1

Profile

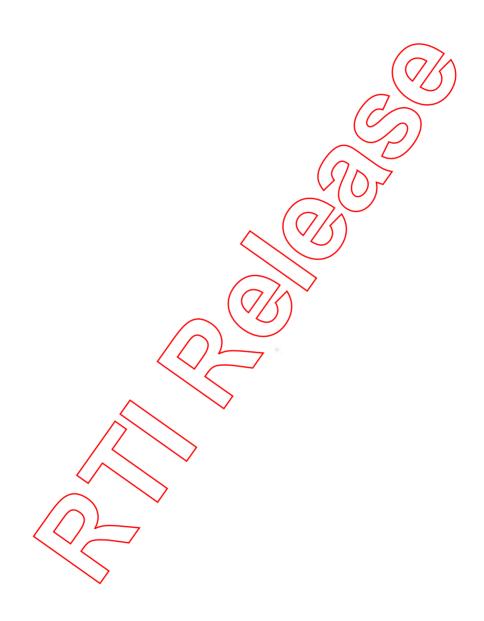
Ċycle	Cycle Point					
Hold @ 95°C, 9min 0s						
Cycling (45 repeats)	tep 1: Hold @ 94°C, 30s					
	Step 2: Hold @ 60°C, 30s					
	Step 3: Hold @ 72°C, 30s, acquiring to Cycling A([Green][1][1])					
Melt	Ramp from 76°C to 89°C					
	Hold for 90s on the 1st step					
	Hold for 5s on next steps,Melt A([Green][1][1])					

Raw Data For Cycling A.Green



No	Color	Name	Туре	Ct	Ct Comment Given Conc (copies/ul) Calc Conc
			Control	21	
47		Sheep Low Positive Control	Positive Control	21.43	
48		Sheep Negative Control	Negative Control	42.94	

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Forensic and Scientific Services **HSSA** | Health Services Support Agency

CERTIFICATE OF ANALYSIS

CLIENT:

Peter Ryan

Department of Agriculture, Investigations and Enforcement

42-44 Qantas Drive Eagle Farm QLD 4009 Package Number

: 14040067

Client Project Client Batch Reference : NERB00542-NERB00582

Meat Species - Jade 1586

Date Received Date Commenced Laboratory Number/s

15 April 2014

: 15 April 2014 : 13PD275-13PD281

FOOD ACT 2006 Section 232

I, the undersigned, a State Analyst, duly appointed under the Food Act 2006, do solemnly and sincerely declare that:-

1. On 15 April 2014, I received the following items from Feter Ryan, Department of Agriculture, Investigations and Enforcement legal receipt No. 5642

Seal Numbers

NERB00542, NERB00549, NERB00567/ NERB00572, NERB00577, NERB00578,

NERB00582

Description

Samples for Species DNA found in Table 1.0 from Jade 1586

2. On 15 April 2014, analysis was commenced in the Molecular Chemistry Laboratory of QHFSS.

Sample Instructions: The samples were submitted querying the presence of species DNA for Beef, Pork, Lamb, and Chicken. The sample format submitted was in these instances, a mixture of packaged products, dumplings, fish ham and fish cakes.

Table 1.0

Client Ref. N	0. <	Lab No.	Sample Description Test Required	Results
NERB00542		13PD275	x 1400 g packet of Narae vegetable dumpling with picture of meat in the bettom left hand corner of the packaging taken from box 15 (blue tape)	Pork (L)
NERB00549	1/	13PD276	1 x 1400 g packet of Narae Kirnchi dumpling with green strip at the top and bottom and red banner on packaging taken from box 22 (red tape)	Pork (L)
NERB00557		13PD277	1 x 1300 g packet of Narae Kun vegetable dumpling with yellow packaging taken from box 30 (yellow tape)	Pork (L)
NER800572	1	13PD278	1 x 500g Sajo Daerim Smoked Fish Ham with orange label with green banner on packaging ACF6EA9HS	Pork (L), Chicken (L)*
NERB00577	/	13PD279	1 x 1kg Saje Daerim Smoked Fish Ham with orange label with green banner on packaging ACF6EA9HS	Pork (L), Chicken (L)*.
NERB00578	1	13PD260	1 x 210g packet of Sajo Daerim fried fish cake (Saecham) on sticks (three individual fish cakes per packet) ACFTCTPLG	Pork (L), Chicken (L)

12/10/15. 55c

13PD275-13PD281

This report overrides all previous reports. The results relate solely to the sample/s as received and are limited to the specific tests undertaken as listed on the report. The results of this report are confidented and are not to be used or disclosed to any other person or used for any other purpose, whether directly or indirectly, unless that use is disclosed or the purpose as expressly authorized in writing by Quisensland Health and the named recipient on this report. To the fullest extent permitted by taw, Quietrusland Fleelith will not be listed for any loss or claim (including legal costs calculated or an independ) basis) which arise because of (a) problems related to the merchantiability, filness or quality of the sample/s, or (b) any negligant or unlawful act or omissions by Quiencland Health under this agreement (including the timing and/or method under which the sample/s were taken, stored or transported).

Enquires Glerin Grahein

39 Kessels Road

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Phone

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Archerfield QLD 4108 AUSTRALIA

+61 7) 3000 9828 FSS@health gld gov gu

	40000004	1 x 1400g packet of N	area Kimehi dumalina y	with roof bannar and	
NER800582	1350201	A 1-15 SOUTH SOUTH VILL	mad taltious annihing i	water for brothings detail	1. Pork (L)
		green top and bettom	of packaging ACF6F9I	NIC.	
				4150	

- 4. Method of Analysis: The samples were assigned a QHFSS Food Chemistry Laboratory number 13PD275-13PD281. The samples were treated by extraction of genomic DNA, by standard methods -QIAGEN DNeasy DNA Tissue Extraction kit Ref. No. 139293717 (QHFSS Quality Information System No. 25335). Extracts were quantified by spectrophotometry for quality and quantity and analysed by Real Time Polymerase Chain Reaction for the presence of DNA from, Bos taurus (Beef), Ovis aries (Lamb), Sus scrofa (Pork), and Gallus gallus (Chicken) by standard method (QHFSS Quality Information System no. 25336) with a limit of detection (LOD) of 0.01%. Melt Curve analysis was undertaken to confirm the identity of the PCR results.
- 5. Remarks: The results of sample 13PD275 13PD281 are found in Table 1. The results are indicated for the presence of DNA from the species listed for analysis. ND (Not Detected) denotes that none of the species listed for analysis were detected. (H) denotes the presence of DNA at a concentration over 1% w/w ± 0.01% and (L) denotes the presence of DNA at a concentration under 1% w/w ± 0.01%. * denotes that the species DNA signal for these products may contain an admixture of other, unidentified species. as provided by the melt curve analysis.

Glenn Graham PhD

State Analyst, Food Chemistry Laboratory

1 May 2014



This report evernices all previous reports. The results relate solely to the sample/s as received and are limited to the specific tests undertaken as listed on the report. The results of this report are confidential and are not to be used or disclosed to any other purpose, whether directly or indirectly, unless that use is disclosed or the purpose is expressly authorised in writing by Quisensland Health and the named recipient on this report. To the fullest extent permitted by two, Quisensland Health will not be liable for any tass or daim (including legal costs calculated on an indominity basis) which ense because of (a) problems related to the merchantability, fitness or quality of the sample/s, or (b) any regigent or untawful act or omissions by Quisensland Health that is connected with any activities or services granded by Quisensland Health under this agreement (including the timing and/or method under which the sample/s were taken, stored or transported). PO Box 594

Enquiries Glenn Graham

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Fav

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NATA Accredited Laboratory

National Association of Testing Authorities, Australia (ABN 59 004 379 748)

has accredited

Queensland Health Forensic and Scientific Services

following demonstration of its technical competence to operate in accordance with

ISO/IEC 17025

This facility is accredited in the field of

Chemical Testing

for the tests shown on the Scope of Accreditation issued by NATA



Alan Patterson Chief Executive

Date of accreditation: 28 June 1951

Accreditation number: 41

Corporate site number: 34



NATA is Australia's government-endorsed laboratory accreditor, and a leader in accreditation internationally.

NATA is a signatory to the international mutual recognition arrangements of the international Laboratory

Accreditation Cooperation (ILAC) and the Asia Pacific Laboratory Accreditation Cooperation (APLAC).

National Association of Testing Authorities, Australia



29 September 2014

Ms H Gregg Principal Quality Advisor Queensland Health 39 Kessels Road COOPERS PLAINS QLD 4108

Dear Ms Gregg

ACCREDITATION NO.	41	t	_	/))		
FACILITY NAME:	Queensland Health	_	_	_	_				
SITE NAME:	Forensic and Scientific Services		<u> </u>	7					
FIELD:	Chemical Testing		7	/					-

Thank you for your submissions, received 10 January, 21 March, 81 March, 2 May, 7 May, 13 May, 28 August and 1 September 2014, provided in response to the reassessment conducted at the above facility during November 2013 and February 2014, and the variation visit conducted in March 2014. At of the conditions raised in the reports on these visits have now been satisfactorily addressed. I am pleased to inform you that the accreditation of your facility in the field of Chemical Testing has been continued.

You are reminded that continuance of accreditation is dependent on adherence to the NATA Rules and the current NATA Accreditation Criteria.

Should the Members Portal have been utilised in relation to this activity, please ensure that you have copies of documents lodged in the 'My Jobs' folder for your permanent records. The associated job folder and its contents will be removed in due course.

Scope of Accreditation

The scope of accreditation has been updated as detailed in the reports on assessment for the above visits. The ambient air sampling and testing has also been consolidated under plass of test 7.65 Air as per policy in Chemical Testing.

A hard copy of your current scope of accreditation, dated 29 September 2014, will be forwarded to you by post.

Please note that as previously advised and as outlined in the NATA Fee Schedule, the facility will be charged for the variation visit component of these activities. Invoices will be forwarded separately and can be identified by the job number 51412.

AP8-1-25-Accreditation-Notification-Letter-Reassessment.doc Reassessment Notification Letter Reassessment (AP8-1-25) / issue 8/ September 2014

Page 1 of 3

/ Legal B Frame NaW 2108 F O Box 7607 Sales year NBW 3108 1 or 2 9728 5222 Francial 1908 523 508

VICTORIA & TASARAMA Level I 675 Victoria Silveri Addominist VIC 3057 Sei 03 9774 6200

CURRENT AND STREET, Post Amenty GR 41th P 0 5 r 11th And of the Try 70 b 41th 1 m 10 1 1700 SCRUTH AUSTRALIA & NT Unit 1 13 King Philam Hoad Uning SA Sold Tel 00 8179 3400

WESTERN ASSOCIATION Business Certina Solic 7 24 Divisio Feet Drive Desired WA 1922 Feet 00 MERCHOOD

The Council's membership includes elected members from the Authorised Representatives. Members are elected for a three year term and as an Authorised Representative you are eligible for election to future Councils.

On behalf of NATA, may I take this opportunity to thank you and your staff for the assistance and hospitality during the recent visit to your facility. If you have any queries with the information contained in this letter please contact Jason Magee at our Brisbane office.

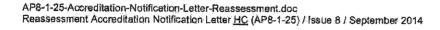
Yours sincerely

For

Jennifer Evans

CHIEF EXECUTIVE OFFICER

Encl. Scope of accreditation dated 29 September 2014



Scope of Accreditation I



.04 Meat and meat products

Analysis of meat and meat products including minced meat, sausages, smallgoods and canned meats for the following determinations by the methods of -

Ash content by classical techniques: AOAC 923.03

Fat content by classical techniques: In-house method 12648 and 26960

Moisture by classical techniques: AOAC 950.46

Protein (Kjeldahl) by classical techniques: In-house method 12643 and 27673

.05 Fish, crustaceans and molluscs

listed as determination(s) by technique(s) using method(s) -

Ash content by classical using AOAC 923.03

Arsenate, arsenite, arsenobetaine, dimethylarsininc acid and monomethylarsonic acid by LC-ICPMS using

in-house method 27644

Fat content as fatty acid methyl ester by GC using in-house method 12634

Histamine by capillary electrophoresis using in-house method 12645

lodine by ICPMS using in house 28218

Ethylmercury, Inorganic mercury and methylmercury by LordPMS lising in-house method 30864

Moisture by classical using AOAC 952.08

Protein (Kjeldahl) by classical using in-house method 12643 and 27673

.06 Sugar and sugar products

for the following determinations by the methods of -

Ash content by classical techniques: AOAC 920.181

Fat content by GC techniques: In-house method 12647

Fructose; glucose; sucrose by HPLC techniques: /rr-hpuse method 12677

Moisture by classical techniques: AQAC 926.45

Protein (Kjeldahl) by classical techniques; In-house method 12643 and 27673

.08 Fruit, jams and other fruit products

for the following determinations by the methods of -

Ash content by classical techniques; AQAC 940.26

Fructose; glucose; sucrose by HPLC techniques: In-house method 12677

Moisture by classical techniques: AOAC \$34.06

.09 Vegetables and vegetable products

for the following determinations by the methods of -

Ash content by classical techniques: AOAC 923.03

Fructose; glucose; lactose; maltose; sucrose by HPLC techniques: AOAC 982.14

Moisture by classical techniques: AOAC 925.10; 929.09

.10 Alcoholic beverages

for the following determinations by the methods of -

Ethanol by GC techniques: In-house method 12669

Sulphate by AES (ICP) techniques: In-house method 24224

Volatile acidity by classical techniques: In-house method 12675

.11 Soft drinks and cordials

for the following determinations by the methods of -

Fructose; glucose; sucrose by HPLC techniques: In-house method 12677

.12 Fruit juices and concentrates

for the following determinations by the methods of -

Ash content by classical techniques: AOAC 923.03

Fructose; glucose; sucrose by HPLC techniques: In-house method 12677

.13 Edible fats and oils

for the following determinations by the methods of -

Fat content as fatty acid methyl ester by GC techniques: In-house method 12634

Free fatty acid value by classical techniques: In-house method 12681

Moisture by classical techniques: AS 2300.1.1

Peroxide value by classical techniques: In-house method 12683

.14 Margarine

Scope of Accreditation N



Analysis of dipping solutions

for the following determinations by the methods of -

Dimethoate; fenthion by HPLC techniques: In-house method 16299

7.65 Air

.02 Ambient air

for the following determinations by the methods of -

Acetaldehyde; acetone; acrolein; benzaldehyde; 2-butanone; butyraldehyde; crotonaldehyde; formaldehyde; hexaldehyde; methacrolein; propionaldehyde; p-tolualdehyde; valeraldehyde by HPLC techniques: USEPA method TO11A and in-house method 21664

Volatile organic compounds by GC-MS techniques: USEPA methods 1014A, 1015 and TO17

.71 Sampling

for the following by the methods of -

Sampling for Volatile organic compounds (VOC): USEPA method TO-11A, TO-14A, TO-15 and TO-17 Sampling using ATD and canister for Volatile organic compounds (VOC): In-house methods 13010, 13016

7.66 Waters

.01 Waters for potable and domestic purposes

Analysis of potable waters, including packaged waters and prineral waters

for the following determinations by the methods of -

Alkalinity by classical technique: In-house method 18298

Arsenate, arsenite, arsenobetaine, dimethylarsining acid and monomethylarsonic acid by LC-ICPMS using

in-house method 27644

Bromate by IC: In-house method 18328

Bromide by IC: In-house method 18204

Chloramines by spectrophotometric technique: In-house method 18202

Chlorate by IC technique: In-house method 18328

Chloride by IC technique: In-house method 18204

Chlorine - free, total by spectrophotometric techniques: In-house method 18202

Chlorite by IC technique: In-house method 18328

Chlorophyll a by spectrophotometric teshniques: In-house method 16623

Colour by spectrophotometric techniques: In-house method 18206

Conductivity by classical echniques: In-house method 18320

Cyanide by classical techniques: In bouse method 18298

EDTA by HPLC: In house method 26,675

Elements as listed under 7.84.01 by AES (ICP), IC, ICP-MS, ISE and XRF techniques: In-house methods

18196, 18207, 18229 and 18236

Endocrine disrupting compounds as listed under 7.84.99 by GCMS using in house 25391

Ethylmercury, Inorganic mercury and methylmercury by LC-ICPMS using in-house method 30864

Fluoride by/IC/and/ISE techniques: In-house method 18207 and 18328

Haloacetic acids by GC/EQD techniques: In-house method 16307

lodide by IC: In-house method 18204

Lignins by spectrophotometric techniques: In-house method 18322

Methylene blue active substances by spectrophotometric techniques: In-house method 16641

Nitrate by IC techniques: In-house method 18204 Nitrite by IC technique: In-house method 18328

Nutrients as listed under 7.84.51 by autoanalyser techniques: In-house methods

Oil and grease by classical techniques: In-house method 16308

Organic matter by classical techniques: In-house 18304

Organics as listed under 7.84 by GC techniques: In-house methods

PCPPs as listed under 7.84.99 by LCMSMS using in house 27701

Perchlorate by IC technique: In-house method 18328

pH by classical techniques: In-house methods 18208 and 18226

Phosphate by IC technique: In-house method 18328

Silica by AES (ICP) techniques: In-house method 18196

Solids - suspended by classical techniques: In-house methods 18211 Solids - total dissolved by classical techniques: In-house methods 18215

Sulfate by IC techniques: In-house method 18204

Scope of Accreditation N



Solids - total dissolved by classical techniques: In-house methods 18215

Sulfate by IC techniques: In-house method 18204

Sulfide by classical techniques: In-house method 18303

Tannins by spectrophotometric techniques: In-house method 18322

Temperature by classical techniques: In-house method 18209

Total organic carbon by classical techniques: In-house method 16628 Trihalomethanes by GC/ECD techniques: In-house method 16306

Turbidity by spectrophotometric techniques: In-house method 18212

Determined by calculation

for the following determinations by the methods of -

Alkalinity - residual: In-house method 18209

Bicarbonate: In-house method 18209 Carbonate: In-house method 18209

Chemical hardness: In-house method 18209

Figure of merit: In-house method 18209

Hardness - temporary, total: In-house method 18209

Hydroxide: In-house method 18209

Mole ratio: In-house method 18209

pHs: In-house method 18209

Saturation index: In-house method 18209

Solids - total dissolved: In-house method 18209

Sodium absorption ratio: In-house method 18209

Total dissolved ions: In-house method 18209

.03 Waters for industrial and steam-raising purposes

for the following determinations by the methods of

Alkalinity by classical technique: In-house method 18208

Bromide by IC: In-house method 1820/4

Chloramines by spectrophotometric technique: In-house method 18202

Chloride by IC technique: In-house method 18204

Chlorine - free, total by spectrophotometric techniques: In-house method 18202

Chlorophyll a by spectrophotometric techniques: In-house method 16623

Colour by spectrophotometric techniques: In-house method 18206

Conductivity by classical techniques in-house method 18320

Cyanide by classical techniques: In house method 18298

EDTA by HPLC: In house method 26575

Elements as listed under 7.84.05 by AES (iCP), ICP-MS, ISE and XRF techniques: In-house methods

18196, 18207, 18229 and 18235

Endocrine disrupting compounds as listed under 7.84.99 by GCMS using in house 25391

Fluoride by ISE techniques In-house method 18207

Haloacetic scids by GC/ECD techniques: In-house method 16307

lodide by IC techniques: In-house method 18204

Lignins by spectroshotometric techniques: In-house method 18322

Methylene blue active substances by spectrophotometric techniques: In-house method 16641

Nitrate by IC techniques: In-house method 18204

Nutrients as listed under 7.84.51 by autoanalyser techniques: In-house methods

Oil and grease by classical techniques: In-house method 16308

Organic matter by classical techniques: In-house 18304

Organics as listed under 7.84 by GC techniques: In-house methods

PCPPs as listed under 7.84.99 by LCMSMS using in house 27701

pH by classical techniques: In-house methods 18208 and 18226

Silica by AES (ICP) techniques: In-house method 18196

Solids - suspended by classical techniques: In-house methods 18211

Solids - total dissolved by classical techniques: In-house methods 18215

Sulfate by IC techniques: In-house method 18204

Sulfide by classical techniques: In-house method 18303

Tannins by spectrophotometric techniques: In-house method 18322

Scope of Accreditation N



Trihalomethanes by GC/ECD techniques: In-house method 16306 Turbidity by spectrophotometric techniques: In-house method 18212

Determined by calculation

for the following determinations by the methods of -

Alkalinity - residual: In-house method 18209

Bicarbonate: In-house method 18209 Carbonate: In-house method 18209

Chemical hardness: In-house method 18209 Figure of merit: In-house method 18209

Hardness - temporary, total: In-house method 18209

Hydroxide: In-house method 18209 Mole ratio: In-house method 18209

pHs: In-house method 18209

Saturation index: In-house method 18209

Solids - total dissolved: In-house method 18209 Sodium absorption ratio: In-house method 18209 Total dissolved ions: In-house method 18209

.05 Trade wastes

Analysis of non-potable recycled waste waters and industrial offluent waters

for the following determinations by the methods of -

Alkalinity by classical technique: In-house method 18208

Bromate by IC: In-house method 18328

Bromide by IC: In-house method 18204

Chloramines by spectrophotometric technique: ka-boyse method 18202

Chlorate by IC technique: In-house method 18328 Chloride by IC technique: In-house method 18204

Chlorine - free, total by spectrophotometric techniques: In-house method 18202

Chlorite by IC technique: In-house method 18328

Colour by spectrophotometric techniques: In-house method 18206

Conductivity by classical techniques: In-house method 18320

Cyanide by classical techniques: In-house method 18298

EDTA by HPLC: In house method 26575

Elements as listed under 7.84.01 by AES (ICP), ICP-MS, ISE and XRF techniques: In-house methods

18196, 18207, 18229 and 18235

Endocrine disrupting compounds as listed under 7.84.99 by GCMS using in house 25391

Fluoride by IC and ISE techniques: In-house method 18207 and 18328

Haloacetic ands by GC/ECD techniques: In-house method 16307

lodide by IQ: In-house method 18204

Lignins by spectrophotometric techniques: In-house method 18322

Methylene blue active substances by spectrophotometric techniques: in-house method 16641

Nitrate by IC techniques: In-house method 18204 Nitrite by IC technique. In-house method 18328

Nutrients as listed under 7.84.51 by autoanalyser techniques: In-house methods

Oil and grease by classical techniques: In-house method 16308

Organic matter by classical techniques: In-house 18304

Organics as listed under 7.84 by GC techniques: In-house methods

PCPPs as listed under 7.84.99 by LCMSMS using in house 27701

Perchlorate by IC technique: In-house method 18328

pH by classical techniques: In-house methods 18208 and 18226

Phosphate by IC technique:In-house method 18328

Silica by AES (ICP) techniques: In-house method 18196

Solids - suspended by classical techniques: In-house methods 18211

Solids - total dissolved by classical techniques: In-house methods 18215

Sulfate by IC techniques: In-house method 18204

Sulfide by classical techniques: In-house method 18303

Tannins by spectrophotometric techniques: In-house method 18322



HealthSupport

Queensland Forensic and Scientific Services

Quality Management System Guide

1 Purpose

Quality systems certification (under ISO 9001) and laboratory technical accreditation (under ISO 17025, ISO 15189, ISO 17043 and ISO Guide 34) requires documentation of all policies and procedures that are critical to the proper functioning of Forensic and Scientific Services (FSS).

The purpose of this guide is to provide an "electronic index" to the principal documents in QIS that describe the quality management system as it applies to FSS. Details of the accreditation, certification and approvals held by FSS are detailed in section 7. This section also details the exclusions for certification against ISO 9001.

2 Scope

This guide is designed to be used by all staff at Forensis and Scientific Services, as well as clients and external assessors of the quality management system.

3 Definitions

Nil

4 Organisation overview

Forensic and Scientific Services is part of Community & Scientific Support, a service line of the Health Support Queensland (HSQ). This reporting relationship is available on the HSQ staff website.

Forensic and Scientific Services (FSS) is Australia's most comprehensive forensic and public health science facility. We provide expert analysis, advice, teaching and research as a vital part of the government response to threats to public health and the environment, epidemics, civil emergencies, criminal investigations and coroners' inquiries into reportable deaths.

The Forensic and Scientific Services (FSS) organisational structure is below.





	- forensic skeletal examination		
	- coronial counselling service		
	- coronial tissue donation		
The state of the s	Police Services		
DNA Analysis	DNA profiling		
	court attendance and delivery of expert evidence in court		
	accident and crime scene evidence analysis		
Forensic Chemistry	Illicit drug analysis		
•	Clandestine laboratory analysis and remediation		
	Physical evidence examination		
	Oil spill comparisons		
	Public and Environmental Health		
Organic Chemistry	Testing for organic and pesticide contaminants in recycled and		
	drinking water, biological specimens and foods		
	Air analysis for volatile organic compounds		
	Algae identification and toxin analysis		
	Food testing to determine safety, chemical composition and		
	compliance		
	Molecular testing to determine the identity and country of origin		
of foodstuffs (///			
Inorganic Chemistry	 Testing for metal and inorganic compounds in the environment, 		
	consumer products, biological materials and food		
	■ Environmental monitoring of nutrients		
Microbiology	Food and water microbiology		
	Reference microbiology		
	Molecular epidemiology Reference and research of Leptospirosis		
Virology	Serology diagnostic laboratory		
· o.o.g y	Miolecular diagnostic laboratory		
	Virus culture		
	Research entomology		
Radiation and	radiation assessments for public and environmental health		
Nuclear Science	programs		
	contaminated land assessment and radiological baseline		
	studies		
//) [_	radiation testing of food, water and soil		
	radiation survey meter calibration		
	waste disposal and transport of radioactive substances		
	• radiation safety training		
	 compliance testing and certification of radiation sources, 		
	-		
	equipment and premises. Clinical Forensic Medicine Unit and interpret medical evidence from victims of crime, alleged mination of adult sexual assault victims		

- offenders and examination of adult sexual assault victims
- provide toxicological advice to the Coroner and courts in relation to suspicious deaths and driving matters



	laboratories across the campus to capture, manage, archive and lawfully destroy records in compliance to Public Records Act 2002					
Scientific Support						
Scientific Skills Development Unit	 Facilitates the development of a learning culture across business units at FSS. Responsible for leading the design, developmed implementation and evaluation of competency based trainst programs across FSS. Delivers scientific and related training facilitating and coordinating the provision of other trainst development services facilitates implementation of court training for scient providing evidence in court 					
Quality and Regulatory Compliance	responsible for managing, maintaining and improving all aspects the FSS quality system including accreditation, certification and any regulatory and legislative requirements relating to these issues. The role provides authoritative professional advice and assistance to management, supervisors and employees on quality systems issues					
Research and Development	provides assistance to 1-55 staff with application and approval documentation monitors and reviews project progress and budget assists with collation and submission of grant applications to facilitates training in research and development areas liaises with Legal and Administrative Law Unit (LALU), universities and other government departments for agministration of cross-jurisdictional projects and contracts facilitates Site Specific Assessment for projects requiring ethical approval Facilitates FSS Human Research Ethics Committee					
Information and Research Services	Provides information and research support to FSS services, including literature searches, electronic journals and databases, epcirits (FSS staff publications), access to Australian Standards and lending services					

Address: 39 Kessels Road

Coopers Plains 4108

Fax: +61 7 3274 9001 Phone: +61 7 3274 9119

Postal address: PO Box 594

Archerfield 4108

Website: www.health.qld.gov.au/qhcss/qhss



Orientation and Induction

All new staff participate in a site induction on day one of employment, and are given copies of the Campus Operations Manual (28335). All staff are required to complete their area induction and mandatory training within 3 months of employment.

All FSS staff are required to have current mandatory training. Details of mandatory training requirements are found on the <u>SSDU website</u> (under induction).

Payroll and rostering

FSS staff are supported by Campus Management and Support, who facilitate all employee related issues such as pay enquiries and leave applications. All relevant forms and industrial relations manuals can be found at either the <u>HSQ HR</u> page or at the <u>PARIS</u> home page.

All FSS staff are required to complete an electronic timesheet.

t. Refer to QIS doc 25310.

		Description	QIS#	
	of	neet management Describes the preparation of and completion of	25310	
procedure timesheets for ESS staff		ire timesheets for ESS staff		

Position Descriptions

Each position within FSS has a documented position description, available from Campus Management and Support. Position Descriptions are used in the recruitment and selection process, and for annual performance and development plans (PDP). Further information about the PDP, and links to relevant documentation can be found at http://gheps.health.gld.gov.au/hr/pdp/performance-dev-planning.htm.

Training and Competency of Staff

The Scientific Skills Development Unit (\$800) coordinates the training needs and professional development of FSS staff. Competency is assessed via completion of training modules (23277), or statement of competence (23947) and on-going assessment is through annual performance and development plans (PDP). Hard copy records are kept for each individual. PDP and competency is recorded in the Professional Development module of QIS (29249). The Professional Development module of QIS is also used to record qualifications, experience and other professional development activities such as publications and conference attendance (26208).

The FSS Learning and Development Guidelines (23651) describes the framework for FSS staff learning and development. The effectiveness of training is evaluated as outlined in section 4 of this document, and also through the following:

- Feedback forms
- Debrjef sessions for trainers
- Monthly trainers meetings
- Training delivery plans
- Comments and suggestions for improvements on training modules
- Opportunity to provide feedback in training module
- FSS_Training email
- Evaluations performed by SSDU training coordinator

Continuing Education and Professional Development

The continuing education and professional development of FSS staff is supported by the SSDU and the Information and Research Service.

The SSDU facilitates course and conference attendance. Details are available via their website.

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33350	FSS	Q	Contracts	Business	Outlines	the	roles	and	responsibilities	for
	Rules	à			users, an	d pro	vides t	he bu	siness rules for u	se

7 Testing

Testing performed within FSS is governed by the HSQ Analytical Services Framework 14475.

Regulatory Compliance

FSS holds the following accreditation, certification and approvals;

Certifying Body	Details	Field of Testing	Relevant Lab(s)	Standard/Act
NATA	Accreditation Number 41	Chemical Testing	Organic Chemistry	ISO 17025
NATA	Accreditation Number 41	Forensic Science	DNA Analysis Forensic Chemistry Forensic Texicology	ISO 17025
NATA	Accreditation Number 41	Biological Testing	Microbiology Organic Chemistry	ISO 17025
NATA	Accreditation Number 41	Medical Testing	Forensic Pathology Microbiology Virology Inorganic Chemistry	ISO 15189
NATA	Accreditation Number 41	Reference Materials Producer	Forensic Toxicology	ISO Guide 34
NATA	Accreditation Number 41	Proficiency Testing Scheme Providers	Env Nutrients	ISO 17043
BSI	Certificate Number FS 609174	N/A	Radiation and Nuclear Sciences, Campus Management, Client Services, SSDU, OHS Excludes NATA	ISO 9001
			accredited labs, IT/LIMS support, and services provided externally by Qld Govt	
TGA	License Number 26688	Testing of Therapeutic Goods	Organic Chemistry	Therapeutic Goods Act
Dept of Agriculture	Approval Number Q0322, Q1755, Q1761, Q2Q51	Approval of Place for Quarantine	Various	Quarantine Act
OGTR	Certification Number 275, 276, 277, 1431, and 1432 IBC Number 418	N/A	Various	Gene Technology Act
DPI	Facility Number 51	Animal Ethics	N/A	Code of Practice for Care and Use of Animals for Scientific Purposes
WHO	Accreditation	World Health Organisation	Leptospirosis	
FAO	Accreditation	Food and Agricultural Organisation	Leptospirosis	
OIE	Accreditation	Office of International Epizooties	Leptospirosis	



20089	FSS Infrequent Testing Guideline	Provides a guideline to FSS laboratories that perform infrequent tests, to ensure the competency of the analyst performing the test, and the currency of the methodology used.
30767	FSS standards for Analytical Spreadsheets	Describes the minimum standards to be followed by FSS for analytical spreadsheets

Test methods used by the laboratory are detailed in separate test methods managed in the Quality Information System (QIS). Acceptance criteria for test results are contained in these test methods.

Internal QC

Specific methods contain requirements for internal quality control and rules for their acceptance/rejection.

Reports

Test reports issued by FSS are NATA endorsed where possible. Some labs have decided to only issue endorsed reports when requested by the client, as the lab is unwilling to accept the risk of reports being recalled if found not to comply with NATA requirements.

FSS has received Crown Law advice that electronically signed analyst certificates are admissible as evidence in court proceedings.

Electronic signatures (e-signatures) are stored in Austan and are linked to the individuals unique login. Use of Austan for e-signatures ensures that they are kept secure and prevents unauthorised use, as the signature appearing on the certificate is the login of the person validating the result (F6). Austan also maintains an audit trail for all activities performed in the system. E-signatures shall not be used for offline reports.

Reports issued as part of the ENCT proficiency testing scheme are the property of the participant, and may be used as required by the participant.

The following are procedures specific to reports issued by FSS laboratories;

QIS#	Title	Description
10623	FSS - Laboratory Report	Defines requirements for the format and
	Format and Content	content of laboratory reports issued by FSS
14076	Test Records	Ensures that laboratory records contain the
		appropriate information for all tests and related
		activities and that they are consistently
	//)_	handled in a manner that retains their integrity
		without loss and/or damage
26993	Procedure for authorising staff	Outlines the procedure for authorising staff to
	to release results for NATA	release results for NATA accredited tests
	accredited tests	
18034	FSS Court Testimony and	Describes the process associated with court
	Attendance Requirements	testimony
29024	Use of offline Forensic	Describes the use of offline templates for
	reporting templates	statement of witness and certificate of analysis
	•	for laboratories reporting in the forensic
		division of AUSLAB
29125	Use of offline Public Health	Contains instructions on the use of offline
	reporting templates	reporting templates in FSS Public Health
		laboratories



		Management including, file creation, applying disposal timeframes, managing physical files and recording movement of files
27131	Destruction of QH Records	Describes the process for approving the
	(FSS)	destruction or extension of records retention

10 Purchasing

Purchasing by FSS is governed by the Department of Health policies. Procedures specific to purchasing at FSS are detailed below;

QIS#	Title	Description				
	1					
10628	Procedure for engaging and	Describes how to assess and record the				
	assessing new vendors	suitability of suppliers/and vendors of				
		consumables, capital items and services				
10622	Verification of Consumables	Describes procedures for ascertaining whether				
	within the Work Group	consumables are nt for purpose. Labelling and				
		record keeping are included.				
		record keeping are included.				
26627	Heurte Acces the EOO					
26627	How to Access the FSS	Outlines the process for contacting the				
	Procurement Advisory Service	Forensic and Scientific Services (FSS)				
		Procurement Advisory Service and the				
		procedure for completing a "Request for				
	_	Quotation" form.				
32164	Minor Works Request	Template to be used where a work area				
		requires minor works to be completed				
32129	Scientific Equipment Service					
<u>04140</u>		Template to be used to request instigation of a				
	Maintenance Agreement (service maintenance agreement for scientific				
	Request	equipment				
32422	Building Plant and Equipment	Template for funding approval, to be used				
	Maintenance Agreement	where a maintenance agreement is required				
	Request	for building related plant and equipment				
27670	FSS Disposal Of Waste	Process for disposal of waste chemicals at				
	Chemicals Procedure	FSS				

11 Equipment

Equipment inventory

An Equipment inventory is available using the Calibration module of the Quality Information System (QIS).

Calibration and Maintanance of Equipment

Calibration and checking intervals are detailed by NATA in the Reference and General Equipment tables. These intervals are reflected in the Calibration module of QIS. Calibration and checking of equipment is supported by other quality assurance activities conducted by the laboratory such as performance of internal quality control checks, routine use of spikes, routine use of reference material, and participation in external quality assurance programs. These additional checks are an immediate indication of the validity of the test results, and an indicator of the correct functioning of the equipment used.

NATA encourages the development of an equipment surveillance program, which moves the emphasis from a reliance on the demonstration of equipment conformance, to a greater contribution from more frequent checks against reference materials, and checking of critical features. In the absence of such a program, the NATA requirements are to be followed.



Queensland

Government

		Biosafety Committee (FSS-IBC), which deals with genetic manipulation issues.
10664	FSS - Human Ethics Committee Functions and Terms of Reference	Describes the composition and operation of the Human Ethics Committee
14079	FSS Medical Advisory Committee – Functions and Terms of Reference	Describes the composition and operation of the Committee, whose principal role is to put into place procedures to be followed in the event of an accident, or exposure of staff to bacterial, viral or rickettsial agents, in the FSS PC3 or PC4 suites
10644	Importation of Exctic Viruses	Describes the procedures necessary of import of level 4 viruses

13 Quality Management System

The Quality Management System is coordinated through the QIS (Quality Information System) database. Flowcharts detailing how to use QIS are contained in the Quality Information System user manuals (26207 – 26214, 29249).

Procedures specific to the functioning of the Quality system at FSS are below;

QIS 莽	Title	_	/	Description
26370	Procedure for staff changes in QIS2			Describes the processes to be
			J	followed by FSS staff and the QIS
				admin team to manage staff changes
	((// {)		in QIS
26369	Procedure for adding new docume	nt	S	Provides FSS staff with instructions
	and QIS Administrators			on how to get a new document added
				to QIS, and provides them with
	~ ~ ~			information on their QIS2
				administrators

Quality Indicators /

Current quality indicators for FSS are:

- 1. critical documents current
- critical QQI's actioned in 30 days. Critical QQI's are external complaints, safety issues and clinical incidents
- external agency non-conformances actioned in 30 days

Document Control

The Procedure for Document Management 10003 outlines the control, development, publication, review/amendment and withdrawal of all documents. Documents are classified according to the hierarchy as detailed in the HSQ Document Hierarchy, Document Types and Document Authorisation Policy 24756. The recommended review period for FSS documents is 24 months.

QIS allows for access to documents to be limited through the private viewers functionality. The use of this functionality to protect the content of documents is not encouraged by HSQ, however it is recognised that FSS deals with issues of an extremely sensitive nature, and the protection of documents is necessary in some cases. Authorisation for protection of

management review) are provided by the FSS Quality Advisor to FSS Executive, which can provide the report to their respective work units.

The Quality Community of Practice, organised by the FSS Quality Advisor, regularly reviews overdue QIS events in each area.

Management review is also part of regular team meetings, which are conducted in accordance with the document Requirements for Team Meetings 17577

Client Feedback

FSS strives to actively seek feedback, both positive and negative, from its clients. This is facilitated through the Scientific Services Liaison Unit (28520). Feedback is sought both formally and informally, electronically (through the use of email and phone meetings), and face to face (via meetings, conferences, working groups etc). Minutes, issues logs, and OQI's are used to record client feedback.

14 Amendment History

Version	Date	Updated by	Amendments
0-13	Various	Various	See archived versions
14	Feb 2015	H Gregg	Clarification of ISO 9001 certification /n/section 7
15	Apr 2015	H Gregg	Statement on use of PT reports added to 'reports' section
16	Sept 2015	H Gregg	Updated OHS sections to reflect organisational restructure. Updated PT participation interval in
			section 7 Updated to new template Removed table of contents

