

December 2013

DNeasy® mericon® 96 QIAcube® HT Kit Handbook

For the automated purification of total nucleic acids from a range of raw and processed food sample types using the QIAcube HT or QIAxtractor® instrument



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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Kit Contents

DNeasy mericon 96 QIAcube HT Kit	(5)
Catalog no.	69571
Number of preps	480
DNeasy 96 plate	5
Food Lysis Buffer*	4 x 200 ml
Proteinase K	1 x 2ml
Buffer PB†	2 x 100 ml
Buffer AW2‡ (concentrate)	1 x 66 ml
Buffer AW2‡ (concentrate)	1 x 13 ml
Buffer EB	2 x 55 ml
TopElute Fluid	1 x 60 ml
Quick-Start Protocol	1

CAUTION:* Contains cetyltrimethylammonium bromide (CTAB): Dangerous for the environment. See page 6 for safety information.

CAUTION:† Contains a chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 6 for safety information.

‡ Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution.

QIAcube HT plasticware	(480)
Catalog no.	950067
Number of preps	480
S-Blocks	5
Filter-Tips OnCor C	9 x 96
Tape Pad	1
Elution Microtubes RS (EMTR)	5
8-Well Strip Caps for EMTR	120

Storage

The DNeasy *mericon* 96 QIAcube HT Kit components are stable at room temperature (15–25°C) and dry conditions.. The DNeasy *mericon* 96 QIAcube HT Kit can be stored at 2–8°C, but buffers should be re-dissolved at 37°C before use, if precipitates are observed. Ensure that all buffers and DNeasy 96 plates are at room temperature (15–25°C) before use.

The DNeasy *mericon* 96 QIAcube HT Kit contains 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 solution at 2–8°C.

Kit components are stable for 1 year under these conditions without showing reduction in performance and quality.

Intended Use

The DNeasy *mericon* 96 QIAcube HT Kit is intended for molecular biology applications in food, animal feed, and pharmaceutical product testing. This product is not intended 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.

Safety Information

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



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

Buffer PB contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. In case liquid containing this buffer is spilt, clean with suitable laboratory detergent and water.

24-hour emergency information

Chemical emergency or accident assistance is available 24 hours a day from:
CHEMTREC

USA & Canada ■ Tel: 1-800-424-9300

Outside USA & Canada ■ Tel: +1-703-527-3887 (collect calls accepted)

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of DNeasy *mericon* 96 QIAcube HT Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

In a globalized food market with increasing demand for food research and monitoring, there is a need for streamlined testing solutions that are sensitive, accurate, and easy to use with a variety of starting materials.

The *mericon* food testing portfolio is a complete system of sample preparation and assay kits that meet the demands listed above. Based on detection by real-time polymerase chain reaction (PCR), *mericon* sample preparation kits and PCR Assays enable fast and reliable detection of a broad range of pathogens, genetically modified organisms, allergens, and plant and animal matter in food, animal feed, or pharmaceutical products.

The DNeasy *mericon* 96 QIAcube HT Kit is designed for rapid purification of DNA from a variety of raw and processed food matrices, while minimizing the carryover of PCR inhibitors inherent to complex food samples. DNeasy *mericon* purified DNA is ready for use in a real-time PCR using one of the *mericon* PCR Assays.

Principle and procedure

The DNeasy *mericon* 96 QIAcube HT Kit uses modified cetyltrimethylammonium bromide (CTAB) extraction.

The cationic detergent CTAB is widely used for efficient extraction of total nucleic acids from a wide range of tissue types. Depending on the salt conditions, CTAB may complex with nucleic acids (low-salt conditions) or complex with inhibitors, such as polysaccharides, proteins, and plant metabolites (high-salt conditions; as found in the Food Lysis Buffer).

The optimized protocols for the DNeasy *mericon* 96 QIAcube HT Kit use CTAB in combination with Proteinase K to first digest compact tissue and to subsequently precipitate proteins with simultaneous precipitation of other cellular and food-derived inhibitors.

Inhibitors are precipitated by centrifugation, while the extracted DNA remains in solution. In the subsequent chloroform extraction, any remaining CTAB-protein, CTAB-debris, or CTAB-polysaccharide complex not precipitated is removed along with other lipophilic inhibitors into the organic phase. Only the aqueous phase containing the DNA and significantly depleted inhibitors is processed further. This phase is mixed with binding buffer (to adjust binding conditions) and applied to the DNeasy 96 plate. The DNA obtained is ready for use in a downstream *mericon* real-time PCR assay.

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 safety data sheets (SDSs), available from the product supplier.

For all protocols

- Homogenizer (see "Important Notes", page 9)
- Vortexer
- Ethanol (96–100%)*
- Chloroform
- Microcentrifuge tubes (2 ml)
- Microcentrifuge with rotor for 2 ml tubes, capable of attaining 14 000 × g
- Shaking incubator or shaking water bath capable of attaining 60°C.
- Pipets and pipet tips
- QIAcube HT Instrument[†]
- QIAcube HT Software version 4.17.1 or higher
- Reagent troughs

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

[†] To process dedicated QIAcube HT Kits on the QIAxtractor instrument, QIAcube HT Software version 4.17.1 or higher is needed together with the Accessories Pack, QXT see ordering information.

Important Notes

Homogenization

Proper disruption of sample material in the protocols is not only important to facilitate food lysis and liberation of DNA, but is also crucial to guarantee a homogeneous starting material, representative of the whole food product.

In this context, anticipated sensitivity and the amount of sample must be considered. The higher the sensitivity requirements (detection of trace amounts of food DNA, for example, allergens or genetically modified organisms [GMOs]) or the more heterogeneous a food product is (e.g., roughly chopped meats in a sausage), the greater the amount of sample material required for homogenization to allow transfer of an overall representative sample into the procedure. In addition to sample size, sample type is also a deciding factor for the homogenization procedure. Both aspects determine the homogenization device best suited for efficient disruption.

To select the best homogenization device, it should be determined whether the food is soft, hard, or extremely hard. Several options are available for each type of food (see below).

Soft samples (e.g., whole fruits in fruit jams or vegetables)

- Small amount of starting sample: Small knife mill, TissueRuptor®, hand blender
- Large amount of starting sample: Large knife mill

Solid/hard samples (e.g., salami or frozen foods)

- Small amount of starting sample: Small knife mill, TissueLyser LT, TissueLyser II, mortar and pestle
- Large amount of starting sample: Large knife mill

Extremely solid/hard samples (e.g., roots or seeds)

- Small amount of starting sample: Small impact mill, TissueLyser LT, TissueLyser II
- Large amount of starting sample: Large impact mill

Disruption using the TissueRuptor/TissueLyser LT systems

Homogenization using the TissueRuptor or TissueLyser systems is best carried out in combination with freezing the sample in liquid nitrogen. This ensures optimal homogenization even with difficult sample material.

Disruption using the TissueRuptor should be carried out without Food Lysis Buffer after freezing the sample in liquid nitrogen. Alternatively, fresh material, such as fruits or vegetables, can be directly disrupted in Food Lysis Buffer without using liquid nitrogen; however, this may cause shearing of high-molecular-weight DNA. We recommend keeping the disruption time to a minimum, to avoid shearing of genomic DNA. With the TissueLyser systems, fresh material can be directly disrupted in lysis buffer without the use of liquid nitrogen. Alternatively, fresh or frozen samples can also be disrupted without lysis buffer after freezing in liquid nitrogen.

We do not recommend disruption of frozen material in lysis buffer as this can result in low yields and degraded DNA.

Starting material

Do not overload the DNeasy membrane, as this can lead to impaired nucleic acid extraction and/or performance in downstream assays. For samples with very high host nucleic acid contents (e.g., flour, grounded hazelnuts, and peanut butter), use less than the maximum amount of sample recommended in the protocol or pretreatments. In some downstream applications such as PCR and RT-PCR, very high background concentrations of nucleic acids may impair the reaction. Use appropriate controls (e.g., an internal control) to verify successful PCR amplification.

Avoid transferring solid material to the S-Block that could reduce flow through the membrane.

Highly viscous fluids may require a treatment to reduce their viscosity to allow for efficient extraction of DNA. Please contact QIAGEN Technical Services for recommendations.

Avoid repeated thawing and freezing of samples since this may reduce DNA yield and quality.

Preparing reagents

QIAGEN Proteinase K

The DNeasy *mericon* 96 QIAcube HT Kit contains ready-to-use proteinase K supplied in a specially formulated storage buffer. The activity of the proteinase K solution is 600 mAU/ml.

QIAGEN Proteinase K is stable for at least 1 year after delivery when stored at room temperature (15–25°C). To store for more than 1 year or if ambient temperature often exceeds 25°C, we recommend storing proteinase K at 2–8°C.

Buffer AW2

Buffer AW2 is supplied as a concentrate in two different sized bottles. Before using for the first time, 160 ml ethanol (96–100%) or 30 ml must be added, as indicated on the bottle. Tick the check box on the bottle label to indicate that ethanol has been added. Mix well after adding ethanol.

TopElute Fluid

TopElute Fluid is used during elution of nucleic acids from the DNeasy membrane. It enables application of a stable and high vacuum and results in equal eluate volumes. In addition, TopElute Fluid eliminates the formation of drops of elution buffer at the outlet nozzles of the DNeasy 96 plates.

TopElute Fluid will be found as a top layer over the elution buffer. It is inert and has no effects on downstream applications.

TopElute does not evaporate and can be stored in the reagent trough.

Assembling the vacuum chamber

The figure illustrates the assembly of the vacuum chamber when using DNeasy 96 plates.

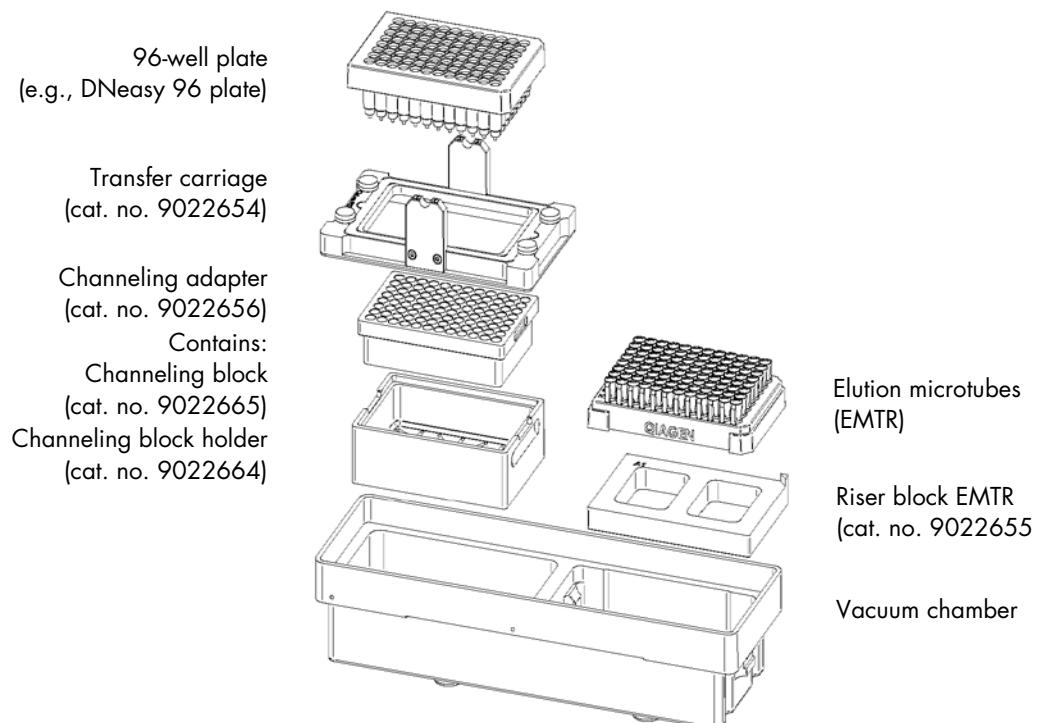


Figure 1. Assembling the vacuum chamber.

All QIAcube HT instruments are delivered with the vacuum chamber components for dedicated QIAcube HT Kits.

Important: If you use a QIAxtractor instrument, ensure that only parts from the Accessories Pack, QXT (black parts) are used. See “Ordering Information”, page 24.

For further information, please refer to the *QIAcube HT User Manual*.

1. Insert the channeling block holder into the left (waste) chamber of the vacuum chamber.
2. Press firmly on the sides of the channeling block holder to seat it in the chamber, sealing the O-ring on the spigot into the drain.
3. Then, place the channeling block into the channeling block holder.
4. Place the DNeasy 96 plate in the transfer carriage. Load the carriage with the DNeasy 96 plate into the left (waste) chamber of the vacuum chamber.

5. Ensure that the carriage is positioned to the left inside the vacuum chamber. Place the riser block EMTR in the right (elution) chamber of the vacuum chamber with the pin of the riser block EMTR in the top right position.
6. Load an elution microtubes rack (EMTR) into the elution chamber.

Optional features

Processing of fewer than 96 samples per run

If processing fewer than 96 samples reuse of DNeasy 96 plates, S-Block and EMTR is possible up to three times.

Note: We recommend using fresh plasticware for every run. In reusing, take extreme care to prevent cross-contamination.

- Store plates in a way that separates the outlet nozzles under the plate, for example, in the S-Block used in the same run or in a fresh 96-well microtiter plate.
- Cover unused wells of the S-Block and DNeasy 96 plate with a tape sheet at all times.
- Remove unused Elution Microtubes from the EMTR in rows of eight tubes.

Off-board lysis protocol

For some applications, it may be necessary to lyse samples in a safety cabinet. A protocol allowing for lysis outside the instrument is available from QIAGEN Technical Services.

Sample data input, data tracking, and LIMS connection

In the software environment information about an item could be seen in the right-hand pane (to open the dialog, click on "A1: Reaction").

See Section 5.11 in the *QIAcube HT User Manual* for more information or ask QIAGEN Technical Service for a detailed example.

Reaction List			
1 Load 200 µl lysis from 96 well plate (vertical) @ B1 into Vacuum Plate (96			
Add...	Edit...	Copy	Delete
Special...	Select All	Up	Down
Reaction Data			
Selected Plate: Vacuum Plate (96 well) @ A1	Hint: Enter elution plate ID here, when vacuum plate is selected.		
Plate ID			
	Import...	Export...	Clear
Well Display Order	<input type="radio"/> Label Sequence	<input checked="" type="radio"/> Pipetting Sequence	
Well	Final Contents	ID	Conc.
A1	200 µl 96 well plate (vertical) @ B1, Well A1		
B1	200 µl 96 well plate (vertical) @ B1, Well B1		
C1	200 µl 96 well plate (vertical) @ B1, Well C1		
D1	200 µl 96 well plate (vertical) @ B1, Well D1		
E1	200 µl 96 well plate (vertical) @ B1, Well E1		
F1	200 µl 96 well plate (vertical) @ B1, Well F1		
G1	200 µl 96 well plate (vertical) @ B1, Well G1		
H1	200 µl 96 well plate (vertical) @ B1, Well H1		
A2			

Figure 2: Example for right-hand pane information.

Sample descriptions can be imported, inserted manually, or inserted using a handheld barcode scanner.

The field "Plate ID" can be used for the unique number that is provided on each EMTR RS plate.

A post-run report is generated for each run and can be used for quality management purposes. It is shown after each run and is automatically saved in the "Reports" subdirectory of the "Data" directory (default location is **C:\Program Files\QIAcubeHT\Data**).

Protocol: Purification of Total DNA from 300 mg Raw and Processed Food or Plant Material

This protocol is designed for the purification of total DNA from a small-scale (300 mg) sample of raw or processed food material, as well as plant material.

Important points before starting

- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- Perform vortexing by pulse-vortexing for 5–10 s.
- Check for precipitates in reagents. If a reagent contains precipitates, incubate at 37°C with gentle shaking to dissolve precipitates. Avoid vigorous shaking which causes foaming.

Things to do before starting

- Homogenize the food sample. For information on disruption procedures and suitable disruption devices, see "Important Notes", page 9.
- Buffer AW2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Ensure all reagents and samples are equilibrated to room temperature (15–25°C).
- Ensure that the relevant version of the **DNeasy 96 mericon QIAcube HT.QSP** run file is installed on the instrument
- QIAcube HT protocol files (file extension ***.QSP**), which contain all the information required to perform a run on the QIAcube HT instrument, are available from www.qiagen.com/p/QIAcubeHT, under the "Resources" tab.
- Ensure that Software version 4.17 or higher is installed. This is mandatory to process DNeasy 96 plates on the QIAcube HT and QIAxtractor.
- Ensure that you are familiar with operating the instrument. Refer to the QIAcube HT User Manual for operating instructions.

Procedure

1. Place the tip discard chute on the worktable so that the chute is over the tip disposal box.

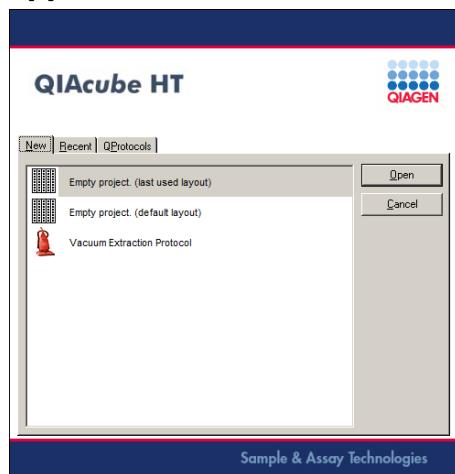
Ensure that the tip discard chute is open and unblocked. Remove the UV protective cap from the tip chute.

Ensure that the tip disposal box is empty and that the opening is aligned with the tip discard chute.

- 2. Switch on the instrument. The switch is located at the back of the instrument, on the lower left.**
- 3. Launch the QIAcube HT Software.**

Note: If the QIAcube HT Software is already open, click  in the toolbar.

- 4. The following screen appears.**



- 5. Select the “QProtocols” tab.**

All Q Protocols that are saved in the appropriate “QProtocols” folder will be listed.

- 6. To open the run file, select the Q Protocol and then click “Open”. Alternatively, double left-click on the Q Protocol**
- 7. A “Protocol Description” of the selected Q Protocol will be displayed and the  icon will appear in the toolbar.**
- 8. Check that the Q Protocol meets your requirements, and then click “Close”.
Note: To view the Q Protocol information box again, click on the  icon in the toolbar.**
- 9. Click  in the toolbar.**

The “Configuration (1)” step of the “Vacuum extraction” wizard opens. This wizard displays protocol parameters. For information about adjusting the parameters, see the *QIAcube HT User Manual*.

10. Select the appropriate number of samples arranged in columns in the 96-well plate. Ensure the “Turn the HEPA filter on automatically” option is checked, and click “Jump to End”.

Reagent and consumable lot numbers can be entered in the “Configuration (1)” window for tracking.

The “Jump to End” button is located at the bottom left of the “Configuration (1)” window.

The “Wizard Summary” window opens. The information in this window can be printed for documentation purposes.

11. Confirm the protocol by clicking “Finish”. The wizard closes.

The QIAcube HT Software calculates the reagent volumes and the number of tips required to complete the protocol. These values are displayed with the worktable layout in the QIAcube HT workspace. For detailed information, see the *QIAcube HT User Manual*.

12. Ensure that there are sufficient numbers of tips for at least all steps until and including lysate transfer, that tip boxes are placed in the indicated positions, and that the lids have been removed from the tip boxes.

Check that the number and position of available and unused tips is the same on the instrument worktable and in the software workspace.

If more tips are required, you will be prompted to replace empty tip racks with new tip racks during the run. Information about the approximate time for refill will be given in the pre-run check. For more information, see the *QIAcube HT User Manual*.

In the software workspace, click on a tip in any tip position to open the “tip info” preview.

13. Prepare the vacuum chamber as described in “Assembling the vacuum chamber”, page 12. See the *QIAcube HT User Manual* for more information.

Note: If fewer than 12 columns (96 wells) are to be processed, seal the unused columns of the DNeasy 96 plate with adhesive tape (supplied). Unused wells must be sealed to ensure proper vacuum operation.

Note: Trim any excess tape.

Note: When reusing DNeasy 96 plate, S-Block, or elution plate, take care to avoid cross-contamination.

Note: Be sure the DNeasy 96 plate is aligned to the left in the carriage and that the carriage is positioned to the left inside the vacuum chamber.

14. Place clean reagent troughs in the indicated positions.

15. With the instrument equipped with all plastic consumables move on to the manual sample pre-treatment.

Manual sample pre-treatment

16. Place 300 mg homogenized food or plant sample in a 2 ml microcentrifuge tube and add 1.5 ml Food Lysis Buffer and 4 µl Proteinase K solution. Vortex briefly to ensure complete mixing and distribution of the sample material.

Note: For samples that swell greatly (e.g., starches), increase the amount of Food Lysis Buffer to ensure that the buffer solution covers the sample material.

17. Incubate in a thermomixer for 30 min at 60°C with constant shaking (1000 rpm). To enhance inhibitor precipitation, cool the sample to room temperature (15–25°C) on ice after incubation.

18. Centrifuge for 5 min at 2500 x g. IMPORTANT: Keep the supernatant.

Note: The volume of supernatant depends on the nature of the applied starting material and the amount of precipitated CTAB-inhibitor complexes. Make sure not to carry over any precipitate from the bottom of the tube into the subsequent protocol steps.

19. Pipet 400 µl chloroform into a fresh 2 ml microcentrifuge tube

Note: Chloroform is a hazardous substance. Always pipet chloroform in a fume hood.

Note: As an organic solvent, chloroform may leak from the pipet tip when transferred from one tube to another. This can be avoided by calibrating the pipet tip to the solvent by repeatedly pipetting up and down before transferring a specific volume.

20. Carefully transfer 550 µl of the clear supernatant from step 17 to the microcentrifuge tube containing the chloroform. Be sure not to carry over material from the bottom phase, which contains precipitated food debris.

Note: The supernatant can be strongly colored. Certain foods may form three phases after centrifugation. If this happens, go through the upper phase with the pipet and transfer only an aliquot of the clear middle phase. If the upper phase has formed a semi-solid film (for example, as observed with chocolate), pierce the film with the pipet and transfer only an aliquot of the clear middle phase.

21. Vortex the microcentrifuge tube vigorously for 15 s and centrifuge at 14,000 x g for 15 min.

Note: If the supernatant is not clear, centrifuge again for 5 min.

- 22. Meanwhile transfer the necessary volumes of all reagents indicated in the QIAcube HT software into the corresponding reagent troughs on the instrument and close the lids.**
- 23. After sample centrifugation, transfer 350 µl of the upper, aqueous phase to the selected S-Block wells. Place the S-Block in the B1 position of the QIAcube HT worktable.**

Automated sample processing

- 24. Start the run immediately by clicking .**

The pre-run check appears.

- 25. Perform the pre-run check.**

Check the state of the worktable items.

Confirm that worktable is setup correctly (instrument does not perform checks for all items). Check the box to the left of the items. A pre-run report can be saved for documentation purposes by clicking .

- 26. After completing the pre-run check, close the instrument hood and click "OK".**

The "OK" button is disabled until all pre-run check items have been checked.

- 27. Click "Cancel" when the "Save as" dialog box appears.**

Optional: Save the run file with a unique file name. See the QIAcube HT User Manual for more details. The protocol run begins.

IMPORTANT: At the beginning of each run an open circuit test and a plate detection test is performed automatically. If the DNeasy 96 plate in the transfer carriage is improperly aligned to the left chamber of the vacuum chamber you will be prompted to place it correctly. After adjusting the position, click "Retry" to initiate the tests again.

- 28. Cover the elution plate (EMTR) with the lid and remove from the elution chamber, when the protocol is complete.**

See the QIAcube HT User Manual for detailed instructions.

Two liquid phases might be found in the Elution Microtubes. If this is the case, TopElute Fluid will be found as a top layer over the elution buffer. It is inert and has no effect on downstream applications.

Cleaning the instrument after completing a run

- 1. Discard racks containing only used tips.**
- 2. Discard leftover reagents.**

Note: We recommend not reusing reagents in multiple runs. Reagents provided are sufficient for at least 5 runs of 96 samples.

Note: Do not clean the trough containing TopElute Fluid with water, but with a dry lint-free cloth only.

- 3. Discard the S-Block or keep partially used blocks for reuse.**
- 4. Remove the transfer carriage and discard the DNeasy 96 plate or keep partially used DNeasy 96 plates for reuse.**
- 5. Clean the carriage, channeling-block, channeling-block holder, and tip chute.**
- 6. With a damp cloth, clean any spilt reagent on the instrument worktable or vacuum chamber.**

Note: For all further cleaning and maintenance operations, see Section 7 of the QIAcube HT User Manual.

- 7. Turn on the UV lamp to decontaminate the worktable by clicking .**

See the QIAcube HT User Manual for detailed instructions.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

Comments and suggestions

Instrument issues

Recovery in case of instrument failure or user interruption

The QIAcube HT interrupts a run upon opening of the hood. The run will proceed normally once the hood is closed. To ensure process safety, this incident is reported in the post-run report.

Instrument failure/cancelled run

It is possible to restart the protocol from the last successful step. The post-run report indicates the step where the error occurred. It is often possible to delete all steps before the indicated step in the right-hand pane and to restart the run from this point. Be sure that all parts and buffers are in the correct position.

Blocked membranes

If liquid is still visible after vacuum steps, remove 500 µl using a pipet and then scrape the surface of the membrane with a fresh pipet tip to relocate any solid particles that may block the membrane. Take care not to damage the membrane. If there is still no liquid flow, pipet all liquid from the well and proceed with the run.

After adding Buffer AW2, open the hood to pause the run. Check if the well is still blocked. If so, remove all liquid using a pipet and mark well as invalid.

We do not recommend perforating the membrane. Uncovered perforated wells will disturb vacuum integrity during elution across the whole plate.

Comments and suggestions

Solid film formed on the lysis solution after incubation at 60°C and subsequent centrifugation

- a) Liberated food components are deposited or compacted on top of the reaction solution after lysis
- Continue with the protocol and pierce any top layer with the pipet. Carefully draw the 550 µl aliquot from the clear middle phase, making sure that the pipet tip is not blocked by food deposits.

No supernatant from which to draw the 550 µl aliquot after incubation at 60°C and subsequent centrifugation

- a) Insufficient food disruption and subsequent swelling of food (e.g., cornflakes)
- Make sure that the food is completely homogenized before adding the Food Lysis Buffer.
- b) Strong swelling of already homogenized food (e.g., starches)
- Apply the same amount of sample, but double the amount of Food Lysis Buffer.

DNA eluate is colored

- a) Inhibitor carryover
- See "DNA does not perform well in downstream experiments", below.

Low DNA yield

- a) Insufficient disruption
- Ensure that the starting material is completely disrupted. See "Disruption using the TissueRuptor/TissueLyser LT systems", page 10.
- b) Insufficient lysis
- Reduce the amount of starting material and/or increase the amount of Food Lysis Buffer.
- Check that the correct amount of Proteinase K has been added to the lysis reaction. If necessary, extend incubation time at 60°C for Proteinase K digest to 90 min and/or increase the amount of Proteinase K to 10 µl.
- c) Buffer AW2 prepared incorrectly
- Make sure that ethanol has been added to Buffer AW2 before use (see "Things to do before starting", page 15).

Comments and suggestions

DNA does not perform well in downstream experiments

- a) Inhibitor carryover A possible inhibitor carryover is sometimes, although not necessarily, identified by a colored eluate. Dilute the sample at least 1:10 before PCR analysis.
- b) Salt carryover Ensure that Buffer AW2 has been used at room temperature (15–25°C).
- c) Insufficient or excess DNA used in downstream application Optimize the amount of DNA used in the downstream application, if necessary. Downstream applications can be adversely affected by insufficient or excess DNA.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
DNeasy mericon 96 QIAcube HT Kit (5)	For 480 preps: DNeasy 96 plates, QIAGEN Proteinase K, Buffers	69571
QIAcube HT plasticware	For 480 preps: 5 S-Blocks, 5 EMTR RS, 2 x 50 Caps for EMTR, 9 x 96 Filter-Tips OnCor C, TapePad	950067
Elution Microtubes RS	24 x 96 Elution Microtubes, racks of 96; includes cap strips	120008
S-Blocks	24 x 96-well blocks with 2.2 ml wells	19585
TissueLyser		
TissueLyser II*	Bead mill, 100-120/220-240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 (available separately)*	85300

* The TissueLyser II must be used in combination with the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96.

Product	Contents	Cat. no.
TissueLyser Adapter Set 2 x 24	2 sets of adapter plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser II	69982
TissueLyser Adapter Set 2 x 96	2 sets of adapter plates for use with Collection Microtubes (racked) on the TissueLyser II	69984
TissueLyser LT*	Compact bead mill, 100-240 V AC, 50–60 Hz; requires the TissueLyser LT Adapter, 12-Tube (available separately)*	85600
TissueLyser LT Adapter, 12-Tube	Adapter for disruption of up to 12 samples in 2 ml microcentrifuge tubes on the TissueLyser LT	69980
Stainless Steel Beads, 5 mm (200)	200 stainless steel beads (5 mm diameter), suitable for use with TissueLyser systems	69989

* The TissueLyser LT must be used in combination with the TissueLyser LT Adapter, 12-Tube.

Product	Contents	Cat. no.
QIAcube HT instrument		
QIAcube HT system	Robotic workstation with UV lamp, HEPA filter, laptop, QIAcube HT operating software, start-up pack, installation and training, 1-year warranty on parts and labor	9001793
Accessories Pack, QXT	Upgrade kit for QIAxtractor instrument; Adapter set to use dedicated QIAcube HT kits on the QIAxtractor Contains: Transfer Carriage (9022654), Riser Block EMTR (9022655) and Channeling Adapter (9022656)	9022649
mericon sample preparation kits		
DNeasy mericon Food Kit (50)	50 QIAquick® Spin Columns, Proteinase K, buffers	69514
mericon DNA Bacteria Kit (100)	Fast Lysis Buffer	69525
mericon DNA Bacteria Plus Kit (50)	50 Pathogen Lysis Tubes L, Fast Lysis Buffer	69534

Product	Contents	Cat. no.
QIAsymphony <i>mericon</i> Bacteria Kit (360)	For 360 preparations: 2 Reagent Cartridges, Piercing Lid, TopElute Fluid (60 ml), Reuse Seal Set	931156
<i>mericon</i> assay kits		
<i>mericon</i> Salmonella spp Kit (24)*	For 24 reactions: <i>mericon</i> Salmonella Assay, Positive Control, Multiplex PCR Master Mix, QuantiTect® Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX™ Dye Solution	290013
<i>mericon</i> L. monocytogenes Kit (24)*	For 24 reactions: <i>mericon</i> L. monocytogenes Assay, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290023

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon Listeria spp Kit (24)*</i>	For 24 reactions: <i>mericon Listeria spp Assay, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution</i>	290123
<i>mericon Campylobacter spp Kit (24)*</i>	For 24 reactions: <i>mericon Campylobacter spp. Assay, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution</i>	290033
<i>mericon Campylobacter triple Kit (24)*</i>	For 24 reactions: <i>mericon Campylobacter triple Assay, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution</i>	290043

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon VTEC stx1/2 Kit (24)*</i>	For 24 reactions: <i>mericon VTEC</i> stx1/2 Assay, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290053
<i>mericon Cronobacter spp Kit (24)*</i>	For 24 reactions: <i>mericon</i> Cronobacter spp Assay, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290063
<i>mericon S. aureus Kit (24)*</i>	For 24 reactions: <i>mericon S. aureus</i> Assay, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290073

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon Quant Legionella spp Kit (96)</i>	For 96 reactions: PCR Assay Quant Legionella spp, Standard DNA, Quant Control DNA, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290085
<i>mericon Quant L. pneumophila Kit (96)</i>	For 96 reactions: PCR Assay Quant L. pneumophila, Standard DNA, Quant Control DNA, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290095
<i>mericon Shigella spp Kit (24)*</i>	For 24 reactions: <i>mericon Shigella</i> spp Assay, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290103

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon Vibrio triple Kit (24)*</i>	For 24 reactions: <i>mericon Vibrio triple Assay</i> , Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290133
<i>mericon Y. enterocolitica Kit (24)*</i>	For 24 reactions: <i>mericon Y. enterocolitica Assay</i> , Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	290113
<i>mericon GMO detection assays</i>		
<i>mericon Screen 35S Kit (24)*</i>	For 24 reactions: <i>mericon Screen 35S Assay</i> , Positive Control, <i>mericon Master Mix</i> , QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	291013

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon Screen 35S Kit (24)*</i>	For 24 reactions: <i>mericon Screen</i> 35S Assay, Positive Control, <i>mericon</i> Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	291013
<i>mericon Screen Nos Kit (24)*</i>	For 24 reactions: <i>mericon Screen</i> Nos Assay, Positive Control, <i>mericon</i> Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	291043
<i>mericon Screen CTP2-CP4EPSPS Kit (24)*</i>	For 24 reactions: <i>mericon Screen</i> CTP2-CP4EPSPS Assay, Positive Control, <i>mericon</i> Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	291053

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon</i> GMO Screen bar Kit (24)*	For 24 reactions: <i>mericon</i> Screen bar Assay, Positive Control, <i>mericon</i> Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	291023
<i>mericon</i> GMO Screen 35S-pat Kit (24)*	For 24 reactions: <i>mericon</i> Screen 35S-pat Assay, Positive Control, <i>mericon</i> Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	291023
<i>mericon</i> MON 810 Kit (24)*	For 24 reactions: <i>mericon</i> MON 810 Assay, Positive Control, <i>mericon</i> Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	291073

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon Quant MON 810 Kit (48)</i>	PCR Assay MON 810, PCR Assay Reference System, Quantification Control DNA, Standard DNA, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291524
<i>mericon Quant RR Soy Kit (48)</i>	PCR Assay RR Soy, PCR Assay Reference System, Quantification Control DNA, Standard DNA, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291514

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon RR Soy Kit (24)*</i>	For 24 reactions: <i>mericon RR Soy Assay, Internal Control, Positive Control, mericon Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution</i>	291113
<i>mericon Animal and Plant Identification Assays</i>		
<i>mericon Pig Kit (24)*</i>	For 24 reactions: <i>mericon Pig Assay, Internal Control, Positive Control, mericon Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution</i>	292013
<i>mericon Cattle Kit (24)*</i>	For 24 reactions: <i>mericon Cattle Assay, Internal Control, Positive Control, mericon Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution</i>	292023

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon</i> Chicken Kit (24)*	For 24 reactions: <i>mericon</i> Chicken Assay, Internal Control, Positive Control, <i>mericon</i> Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	292033
<i>mericon</i> Goat Kit (24)*	For 24 reactions: <i>mericon</i> Goat Assay, Internal Control, Positive Control, <i>mericon</i> Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	292053
<i>mericon</i> Horse Kit (24)*	For 24 reactions: <i>mericon</i> Horse Assay, Internal Control, Positive Control, <i>mericon</i> Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution	292143

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon Ruminants Kit (24)*</i>	For 24 reactions: <i>mericon Ruminants Assay, Internal Control, Positive Control, mericon Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution</i>	292073
<i>mericon Sheep Kit (24)*</i>	For 24 reactions: <i>mericon Sheep Assay, Internal Control, Positive Control, mericon Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution</i>	292063
<i>mericon Pig Kit (24)*</i>	For 24 reactions: <i>mericon Pig Assay, Internal Control, Positive Control, mericon Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution</i>	292013

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon Turkey Kit (24)*</i>	For 24 reactions: <i>mericon Turkey Assay, Internal Control, Positive Control, mericon Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-free water, 50x ROX Dye Solution</i>	292043
<i>mericon Apricot Kernels Kit (24)</i>	For 24 reactions: PCR Assay Apricot Kernels, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye	293033
<i>mericon Corn Kit (24)*</i>	For 24 reactions: PCR Assay Corn, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	293023

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon Soy Kit (24)*</i>	For 24 reactions: PCR Assay Soy, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	293013

For a complete list of accessories, visit www.qiagen.com/p/QIAcubeHT

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Notes

Notes

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www.qiagen.com

Australia ■ techservice-au@qiagen.com

Austria ■ techservice-at@qiagen.com

Belgium ■ techservice-bnl@qiagen.com

Brazil ■ suportetecnico.brasil@qiagen.com

Canada ■ techservice-ca@qiagen.com

China ■ techservice-cn@qiagen.com

Denmark ■ techservice-nordic@qiagen.com

Finland ■ techservice-nordic@qiagen.com

France ■ techservice-fr@qiagen.com

Germany ■ techservice-de@qiagen.com

Hong Kong ■ techservice-hk@qiagen.com

India ■ techservice-india@qiagen.com

Ireland ■ techservice-uk@qiagen.com

Italy ■ techservice-it@qiagen.com

Japan ■ techservice-jp@qiagen.com

Korea (South) ■ techservice-kr@qiagen.com

Luxembourg ■ techservice-bnl@qiagen.com

Mexico ■ techservice-mx@qiagen.com

The Netherlands ■ techservice-bnl@qiagen.com

Norway ■ techservice-nordic@qiagen.com

Singapore ■ techservice-sg@qiagen.com

Sweden ■ techservice-nordic@qiagen.com

Switzerland ■ techservice-ch@qiagen.com

UK ■ techservice-uk@qiagen.com

USA ■ techservice-us@qiagen.com

