February 2017

QIAamp® DSP DNA FFPE Tissue Kit Handbook

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Version 1

IVD

For in vitro diagnostic use

CE

REF 60404

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R3 MAT 1062689EN

Sample to Insight

QIAGEN

Contents

Intended Use

The QIAamp DSP DNA FFPE Tissue Kit is a system that uses silica-membrane technology (QIAamp technology) for isolation and purification of genomic DNA from formalin-fixed, paraffin-embedded (FFPE) biological specimens.

The product is intended to be used by professional users, such as technicians and physicians, who are trained in molecular biology techniques for in vitro diagnostic (IVD) purposes; it is intended for manual sample preparation purposes and gives no test results, qualitative or quantitative.

Summary and Explanation

The QIAamp DSP DNA FFPE Tissue Kit is used for purification of DNA from FFPE tissue sections. It uses well-established QIAamp DNA Micro technology for purification of genomic and mitochondrial DNA from small sample volumes or sizes. The kit combines the selective binding properties of a silica-based membrane with flexible elution volumes.

Lysis conditions allow genomic DNA to be efficiently purified from FFPE tissue sections without the need for overnight incubation. Incubation at an elevated temperature after Proteinase K digestion partially removes formalin crosslinking of the released DNA, potentially improving yield, as well as DNA performance in downstream assays. Note that DNA isolated from FFPE samples is usually of lower molecular weight than DNA from fresh or frozen samples. The degree of fragmentation depends on the type and age of the sample and the conditions used for fixation.

After sample lysis, the simple QIAamp DSP DNA FFPE Tissue procedure is suited for simultaneous processing of multiple samples.

It is the user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by the QIAGEN performance studies described in the handbook.

Principle of the Procedure

The QIAamp DSP DNA FFPE Tissue procedure consists of six steps (Figure 1):

- Paraffin removal: Paraffin is dissolved in xylene and removed
- Lysis: Sample is lysed at 56°C under denaturing conditions with Proteinase K
- Heat: Incubation at 90°C reverses formalin crosslinking
- Bind: DNA binds to the membrane and contaminants flow through
- Wash: Residual contaminants are washed away
- Elute: Pure, concentrated DNA is eluted from the membrane

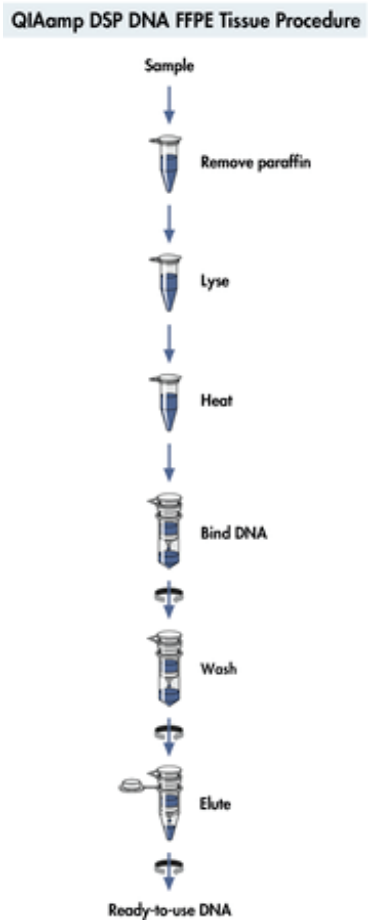

Figure 1. QIAamp DSP DNA FFPE Tissue procedure.

Materials Provided

Kit contents

QIAamp DSP DNA FFPE Tissue Kit			**(50)**
Catalog no.			**60404**
Number of reactions			**50**
QIAamp MinElute®	QIAamp MinElute Columns with Wash Tubes	COL	50
WT	Wash Tubes (2 ml)	WASH TUBE	3 x 50
ET	Elution Tubes (1.5 ml)	ELU TUBE	50
LT	Lysis Tubes (2 ml)	LYS TUBE	50
ATL	Tissue Lysis Buffer	TIS LYS BUF	10 ml
AL	Lysis Buffer*	LYS BUF	12 ml
AW1	Wash Buffer 1* (concentrate)	WASH BUF 1 CONC	19 ml
AW2	Wash Buffer 2† (concentrate)	WASH BUF 2 CONC	13 ml
ATE	Elution Buffer†	ELU BUF	12 ml
PK	Proteinase K	PROTK	1.25 ml
–	Instructions For Use (Handbook)	HB	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 10 for warnings and precautions.

† Contains sodium azide as a preservative.

Materials Required but Not Provided

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.

Reagents

- Xylene
- Ethanol (96–100%)*

Consumables

- If a decision is taken not to use the tubes provided in the kit, we recommend 1.5 ml or 2 ml microcentrifuge tubes (for lysis steps) and 1.5 ml microcentrifuge tubes (for elution steps) (available from Eppendorf® [Safe-Lock: cat. no. 022363204, US; cat. no. 0030 120.086, Europe], or Sarstedt [cat. no. 72.690]). We recommend DNase/RNase-free, conical shape tubes with secure lids.
- Pipets and pipet tips (to avoid cross-contamination, we strongly recommend pipet tips with aerosol barriers)

Equipment

- Thermomixer†, heated orbital incubator, heating block, or water bath capable of incubation at 56°C, 70°C and 90°C
- Microcentrifuge† with rotor for 2 ml tubes
- Vortexer

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

† To ensure that samples are properly processed in the QIAamp DSP DNA FFPE procedures, we strongly recommend that instruments are calibrated according to the manufacturers' recommendations.

Warnings and Precautions

For in vitro diagnostic use

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 AL and Buffer AW1 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled 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 hazard and precautionary statements apply to components of the QIAamp DSP DNA FFPE Tissue Kit.

Buffer AL

Contains: guanidine hydrochloride; maleic acid. Warning! May be harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. May cause an allergic skin reaction. If eye irritation persists: Get medical advice/ attention. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Take off contaminated clothing and wash it before reuse. IF ON SKIN: Wash with plenty of soap and water. If skin irritation occurs: Get medical advice/ attention. Wear protective gloves/ protective clothing/ eye protection/ face protection.

Buffer ATL

Warning! Causes mild skin irritation. If skin irritation occurs: Get medical advice/ attention.

Buffer AW1

Contains: guanidine hydrochloride. Warning! Harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. Call a POISON CENTER or doctor/ physician if you feel unwell. Dispose of contents/ container to an approved waste disposal plant. Take off contaminated clothing and wash it before reuse. Wear protective gloves/ protective clothing/ eye protection/ face protection.

Proteinase K

Contains: Proteinase K. Danger! Causes mild skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray. Dispose of contents/ container to an approved waste disposal plant. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/ physician. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. Wear respiratory protection.

Reagent Storage and Handling

QIAamp MinElute columns should be stored at 2–8°C upon arrival and can be used until the expiration date shown on the kit box.

All buffers can be stored at room temperature (15–25°C) and are stable until the kit expiration date. However, reconstituted Buffer AW1 and AW2 can be stored at room temperature (15–25°C) for up to 1 year or until the expiration date for the kit, whichever is shorter.

The QIAamp DSP DNA FFPE Tissue Kit contains a ready-to-use Proteinase K solution, which is supplied in a specially formulated storage buffer. Proteinase K is stable until the kit expiration date when stored at room temperature (15–25°C).

Specimen Handling and Storage

Standard formalin-fixation and paraffin-embedding procedures should be used. To limit the extent of DNA fragmentation, be sure to:

- Fix tissue samples in formalin as per laboratory protocol (10% neutral buffered formalin is generally accepted) as quickly as possible after surgical removal.
- Use a fixation time of 14–24 hours. Limit fixation times as prolonged fixation (for example >24 hours) may lead to more severe DNA fragmentation, resulting in poor performance in downstream assays).
- Thoroughly dehydrate samples prior to embedding (residual formalin can inhibit the Proteinase K digestion).

DNA is eluted in Buffer ATE and is immediately ready for use in amplification reactions or for storage (conditions dependent upon user requirements). Refer to the relevant kit handbooks for recommended storage conditions for specific QIAGEN downstream applications.

Procedure

Important points before starting

- All reagents supplied in the QIAamp DSP DNA FFPE Tissue Kit are intended to be used solely with the other reagents in the same QIAamp DSP DNA FFPE Tissue Kit. Substitutions to the reagents in the kit must not be made if optimal performance is to be maintained.
- After receiving the kit, check the kit components for damage. If the packs or the buffer bottles are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to "Warnings and Precautions", page 10). Do not use damaged kit components, since their use may lead to poor kit performance.
- Do not use kit components from other kits with the kit you are currently using, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- This kit should only be used by personnel trained in in vitro diagnostic laboratory practice.
- Always wear latex or vinyl gloves while handling reagents and samples to prevent contamination from the surface of the skin or from dusty laboratory equipment. Hands and dust particles may carry bacteria and molds, and are common sources of contamination. Change gloves frequently and keep tubes closed.
- Unused buffers, flow-throughs and sample remains should be disposed according to local procedures.
- If you are using your own plasticware, the use of DNase/RNase-free low binding, disposable polypropylene 1.5–2ml conical tubes with secure lids is recommended throughout the purification procedure.
- Perform all centrifugation steps at room temperature (15–25°C).
- All buffers should be stored at room temperature (15–25°C) and they should be well mixed before use.

- Set a thermomixer or heated orbital incubator to 56°C for use in step 11. If a thermomixer or heated orbital incubator is not available, a heating block or water bath can be used instead.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions below.
- Quality control procedures at QIAGEN employ functional kit release testing for each individual kit lot. Therefore do not mix reagents from different kit lots, and do not combine individual reagents from different reagent lots.

Preparation of buffers

Preparing Buffer ATL

- Before starting the procedure, check whether precipitate has formed in Buffer ATL. If necessary, dissolve by heating to 70°C with gentle agitation.

Preparing Buffer AL

- Before starting the procedure, check whether precipitate has formed in Buffer AL. If necessary, dissolve by heating to 70°C with gentle agitation.

Preparing Buffer AW1

- Add 25 ml ethanol (96–100%) to the bottle containing 19 ml of concentrated Buffer AW1. Check the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 can be stored at room temperature (15–25°C) for up to 1 year or until the expiration date of the kit, whichever is shorter. We recommend writing the reconstitution date on the label of the buffer.

  Note: Before starting the procedure, mix reconstituted Buffer AW1 by shaking.

Preparing Buffer AW2

- Add 30 ml ethanol (96–100%) to the bottle containing 13 ml of concentrated Buffer AW2. Check the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW2 can be stored at room temperature (15–25°C) for up to 1 year or until the expiration date on the kit, whichever is shorter. We recommend writing the reconstitution date on the label of the buffer.

  Note: Before starting the procedure, mix reconstituted Buffer AW2 by shaking.

Starting material

The starting material for DNA purification is cut sections of FFPE tissue (ideally freshly cut). Multiple sections can be combined in one preparation. If you have no information about the nature of your starting material, we recommend starting with no more than three sections per preparation.

The user should optimize the number of sections, section thickness and section surface area for any procedures used in their laboratory. If the kit is being used in conjunction with a QIAGEN downstream application, refer to the relevant handbook for instructions.

Handling procedure to avoid cross-contamination

Due to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling QIAamp MinElute columns to avoid cross-contamination between samples:

- Do not to overfill the tubes with tissue.
- Change the scalpels between samples when scraping the tissue.
- Carefully apply the sample or solution to the QIAamp MinElute column. Pipetting the sample into the QIAamp MinElute column without wetting the rim of the column.

- Always change pipet tips between liquid transfers. We recommend the use of aerosol-barrier pipet tips.
- Always use new wash tubes when performing sample washing steps.
- Ensure tube lids are closed entirely before vortexing and centrifuging.
- Ensure that QIAamp MinElute column is closed entirely before centrifuging.
- After all pulse-vortexing steps and 90°C incubation steps briefly centrifuge the microcentrifuge tubes to remove drops from the inside of the lids.
- Open only one QIAamp MinElute column at a time and take care to avoid generating aerosols.
- Always change the scalpels between samples.
- Always change pipet tips between liquid transfers. To minimize cross-contamination, we recommend the use of aerosol-barrier pipet tips and avoid the use of multistep pipets.
- Always use disposable gloves and regularly check if they may be contaminated with sample material. Discard gloves if you suspect that they have become contaminated.
- Open only one tube at a time.

Centrifugation

QIAamp MinElute columns will fit into most standard 1.5–2 ml microcentrifuge tubes. Additional 2 ml wash tubes are available separately (QIAGEN, cat. no. 19201). Centrifugation of QIAamp MinElute columns is performed at approximately 6000 x *g* to reduce centrifuge noise. Centrifugation at full speed will not improve DNA yields. However, centrifugation of QIAamp MinElute columns at full speed is required in two steps of the procedure: the dry centrifugation step after the membranes are washed and the elution step. Centrifugation at full speed is also required to bring down the sample after the xylene treatment and the ethanol wash step.

All centrifugation steps should be carried out at room temperature (15–25°C). Low centrifugation temperature may lead to suboptimal extraction.

Processing QIAamp MinElute columns in a microcentrifuge

- Always close QIAamp MinElute columns before placing them in the microcentrifuge.
- Avoid touching the QIAamp MinElute column membrane with the pipet tip.
- Flow-through fractions may contain hazardous waste and should be disposed of appropriately.
- For efficient parallel processing of multiple samples, we recommend filling a rack with wash tubes into which QIAamp MinElute columns can be transferred after centrifugation. Used wash tubes containing flow-through can be discarded, and the new wash tubes containing the QIAamp MinElute columns can be placed directly in the microcentrifuge.
- Ensure full sample traceability is kept during entire process.

Eluting purified DNA

For downstream applications that require small starting volumes (e.g., some PCR assays), a more concentrated eluate may increase assay sensitivity but may also result in an increase in concentration of potential inhibitors.

An increase in elution volume will decrease the concentration of DNA in the eluate.

The volume of eluate recovered may be approximately 5 µl less than the volume of Buffer ATE applied to the QIAamp MinElute column. For example, an elution volume of 20 µl results in ≥15 µl eluate. The volume of eluate recovered depends on the nature of the sample.

It is the user's responsibility to optimize the elution volume for any procedures used in their laboratory. Refer to kit handbooks for recommended elution volumes required for specific QIAGEN downstream applications.

Yields may be increased if the column is incubated with Buffer ATE at room temperature for example 5 minutes before centrifugation. Eluted DNA can be collected in the 1.5 ml elution tubes (provided). Storage conditions for the eluted DNA are dependent upon user-defined requirements. Refer to kit handbooks for recommended storage conditions for specific QIAGEN downstream applications.

Protocol: Isolation of Genomic DNA from FFPE Tissue Sections

Procedure

1. Using a scalpel, trim excess paraffin off the sample block.
2. Cut sections following standard laboratory practice (see "Starting material", page 15). The user should optimize the number of sections, section thickness and section surface area for any procedures used in their laboratory. Ensure that traceability of the samples is kept during entire procedure.
3. Immediately scrape the tissue from the sections using sterile scalpel in a Lysis Tube (provided). Make sure all the available tissue is placed in the tube. Add 1 ml xylene to the sample, close the lid and vortex vigorously until paraffin dissolves (e.g., 10 s). Make sure the tube is fully closed to avoid xylene spillage, cross-contamination between samples and possible contact with the xylene.

   Note: Use xylene in fume hoods or other appropriate containment apparatus.
4. Centrifuge at full speed for approximately 2 min at room temperature to collect the tissue pellet. If no tissue pellet was formed repeat this step.

   Note: Low centrifugation temperature may lead to suboptimal extraction.
5. Remove the supernatant by pipetting and discard it. Retain the pellet.

   Supernatant contains xylene which is hazardous waste and should be disposed of appropriately according to local regulations.
6. Add 1 ml ethanol (96–100%) to the tissue pellet, and mix thoroughly by vortexing.

   The ethanol extracts residual xylene from the sample and should be disposed of appropriately.

7. Centrifuge at full speed for approximately 2 min at room temperature.

   Remove the supernatant by pipetting. Do not remove any of the pellet.

   Carefully remove any residual ethanol using a fine pipet tip. Open the tube and incubate at 15–40°C, until all residual ethanol has evaporated. Removal of residual ethanol is crucial for extraction success.

   Note: Lower incubation temperature slows time of evaporation, whereas higher temperature can overdry the pellet making it difficult to suspend.

8. Resuspend the pellet in 180 µl Buffer ATL. Add 20 µl Proteinase K, and mix by vortexing.

   Note: Pellet must be well resuspended in the ATL buffer to ensure the maximum yield recovery.

9. Incubate at 56°C ± 3°C for approximately 1 h (until the sample has been completely lysed).

10. Incubate at 90°C ± 5°C for 1 hour ± 5 min.

    The incubation at 90°C in Buffer ATL partially reverses formaldehyde modification of nucleic acids. Shorter incubation times or lower incubation temperatures may impact DNA quality and quantity. If using only one heating block, leave the sample at room temperature after the 56°C incubation until the heating block has reached 90°C.

11. Briefly centrifuge the tube to remove drops from the inside of the lid.

12. Add 200 µl Buffer AL to the sample and mix thoroughly by vortexing. Then, add 200 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. A white precipitate may form on addition of Buffer AL and ethanol. This precipitate does not interfere with the QIAamp procedure. Always use fresh mix and discard it immediately after use.

13. Briefly centrifuge the tube to remove drops from the inside of the lid.

14. Carefully transfer the entire lysate to the QIAamp MinElute column (in a 2 ml wash tube) without wetting the rim, close the lid, and centrifuge at approximately 6000 x *g* for ≥1 min. Place the QIAamp MinElute column in a clean 2 ml wash tube (provided), and discard the wash tube containing the flow-through.

    If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the QIAamp MinElute column is empty.

15. Carefully open the QIAamp MinElute column and add 500 µl reconstituted Buffer AW1 without wetting the rim. Close the lid and centrifuge at approximately 6000 x *g* for ≥1 min. Place the QIAamp MinElute column in a clean 2 ml wash tube, and discard the wash tube containing the flow-through.

16. Carefully open the QIAamp MinElute column and add 500 µl reconstituted Buffer AW2 without wetting the rim. Close the lid and centrifuge at approximately 6000 x *g* for ≥1 min. Place the QIAamp MinElute column in a clean 2 ml wash tube, and discard the wash tube containing the flow-through.

    Contact between the QIAamp MinElute column and the flow-through should be avoided. Be sure to balance the centrifuge rotor. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains ethanol, coming into contact with the QIAamp MinElute column. Take care when removing the QIAamp MinElute column and wash tube from the rotor, so that flow-through does not come into contact with the QIAamp MinElute column.

17. Centrifuge at full speed (approximately 20,000 x *g*) for approximately 3 min to dry the membrane.

    Ethanol carryover into the eluate may interfere with some downstream applications.

18. Place the QIAamp MinElute column in a clean 1.5 ml elution tube (provided), and discard the wash tube containing the flow-through. Carefully open the lid of the QIAamp MinElute column and apply 20–200 µl Buffer ATE to the center of the membrane.

    IMPORTANT: If using small elution volumes (<50 µl), dispense Buffer ATE onto the center of the membrane to ensure complete elution of bound DNA. QIAamp MinElute columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. The volume of eluate will be approximately 5 µl less than the volume of elution solution applied to the column.

19. Close the lid and incubate at room temperature (15–25°C) for at least 1 min. Centrifuge at full speed (approximately 20,000 x *g*) for ≥1 min.

    Incubating the QIAamp MinElute column loaded with Buffer ATE for approximately 5 min at room temperature before centrifugation may increase DNA yield.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAamp DSP DNA FFPE Tissue Kits is tested against predetermined specifications to ensure consistent product quality.

Limitations

The kit performance has been established using formalin-fixed, paraffin-embedded tissues (FFPE tissues) for isolation of genomic DNA.

It is the user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by the QIAGEN performance studies described in the handbook.

To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used. For further validation, the guidelines of the International Conference on Harmonization of Technical Requirements (ICH) in ICH Q2(R1) Validation Of Analytical Procedures: Text And Methodology are recommended.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Using the QIAamp DSP DNA FFPE Tissue Kit, RNA may be copurified with the DNA if it is present in the sample.

Performance Characteristics

See **www.qiagen.com/QIAamp-DSP-DNA-FFPE-Tissue-CE** for performance characteristics of the QIAamp DSP DNA FFPE Tissue Kit.

Symbols

The following symbols may appear on the packaging and labeling:

Symbol	Symbol definition
 <N>	Contains reagents sufficient for <N> reactions
	Use by
	In vitro diagnostic medical device
	Upon arrival
	Catalog number
	Lot number
	Material number
	Components
	Contains
	Number

Symbol	Symbol definition
	Write down the current date after adding ethanol to the bottle
	Ethanol
	Adding
	Guanidine hydrochloride
	Maleic acid
	Global Trade Item Number
	Temperature limitation
	Manufacturer
	Consult instructions for use
	Caution

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Ordering Information

Product	Contents	Cat. no.
QIAamp DSP DNA FFPE Tissue Kit — for purification of genomic DNA from paraffin-embedded tissues		
QIAamp DSP DNA FFPE Tissue Kit (50)	For 50 DNA preps: 50 QIAamp MinElute® Columns, Proteinase K, Buffers, Wash Tubes (2 ml), Elution Tubes (1.5 ml), Lysis Tubes (2 ml)	60404

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Trademarks: QIAGEN®, Sample to Insight®, QIAamp®, MinElute® (QIAGEN Group); Eppendorf® (Eppendorf AG).

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