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QIAamp® DNA FFPE Tissue Handbook

For purification of genomic DNA from formalin-fixed, paraffin-embedded tissues

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAamp DNA FFPE Tissue Kit	**(50)**
Catalog no.	**56404**
Number of preps	**50**
QIAamp MinElute® Columns	50
Collection Tubes (2 ml) 3 x	50
Buffer ATL	14 ml
Buffer AL*	12 ml
Buffer AW1* (concentrate)	19 m
Buffer AW2† (concentrate)	13 ml
Buffer ATE†	20 ml
Proteinase K	1.25 ml

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 3 for safety information
† Contains sodium azide as a preservative

Storage

QIAamp MinElute columns should be stored at 2–8°C upon arrival and are stable under these conditions for at least one year after delivery, if not otherwise stated on the label. However, short-term storage of up to 4 weeks at room temperature (15–25°C) does not affect performance.

All buffers can be stored at room temperature and are stable for at least one year after delivery.

The QIAamp DNA FFPE Tissue Kit contains a novel, ready-to-use proteinase K solution, which is supplied in a specially formulated storage buffer. Proteinase K is stable for at least one 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.

Intended Use

The QIAamp DNA FFPE Tissue Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

QIAcube® Connect 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 trained in molecular biological techniques and the operation of QIAcube Connect.

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 to 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 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.

Quality Control

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

Introduction

The QIAamp DNA FFPE Tissue Kit is optimized 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 of between 20 and 100 µl.

Specially optimized 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, improving yields, 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 DNA Micro procedure, which is highly suited for simultaneous processing of multiple samples, yields pure DNA in less than 30 minutes.

DNA is eluted in Buffer ATE or water and is immediately ready for use in amplification reactions or for storage at –30°C to –15°C. Purified DNA is free of proteins, nucleases and other impurities.

Principle and procedure

The QIAamp DNA FFPE Tissue procedure consists of 6 steps (see flowchart):

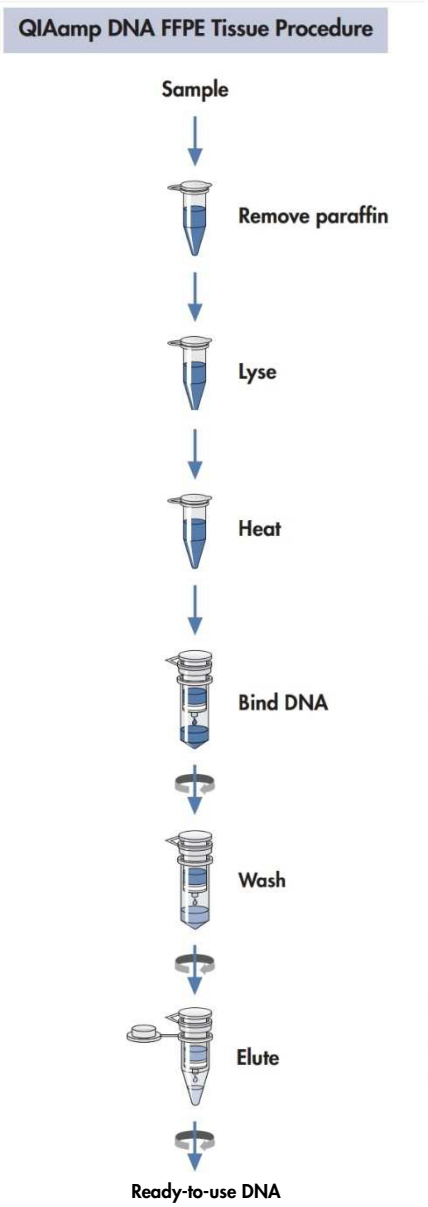
- Remove paraffin: paraffin is dissolved in xylene and removed
- Lyse: sample is lysed under denaturing conditions with proteinase K
- Heat: incubation at 90°C reverses formalin crosslinking
- Bind: DNA binds to the membrane and contaminants flow through
- Wash: residual contaminants are washed away
- Elute: pure, concentrated DNA is eluted from the membrane

Automated purification of DNA on QIAcube® Instruments

Purification of DNA can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), enabling you to continue using the QIAamp DNA FFPE Tissue Kit for purification of high-quality DNA.

QIAcube instruments are preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at **www.qiagen.com/qiacubeprotocols**.

QIAcube Connect.


QIAamp DNA FFPE Tissue Procedure
Sample
Remove paraffin
Lyse
Heat
Bind DNA
Wash
Elute
Ready-to-use DNA

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.

- Xylene
- Ethanol (96–100%)*
- 1.5 ml or 2 ml microcentrifuge tubes (for lysis steps)
- 1.5 ml microcentrifuge tubes (for elution steps) (available from Brinkmann [SafeLock, cat. no. 022363204], Eppendorf [Safe-Lock, cat. no. 0030 120.086] or Sarstedt [Safety Cap, cat. no. 72.690])†
- Pipet tips (to avoid cross-contamination, we recommend pipet tips with aerosol barriers)
- Thermomixer, heated orbital incubator, heating block or water bath capable of incubation at 90°C
- Microcentrifuge with rotor for 2 ml tubes
- Vortexer
- **Optional**: RNase A (100 mg/ml; cat. no. 19101) protocol)

* 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.

Important Notes

Starting material

Standard formalin-fixation and paraffin-embedding procedures always result in significant fragmentation of nucleic acids. To limit the extent of DNA fragmentation, be sure to:

- Fix tissue samples in 4–10% formalin as quickly as possible after surgical removal.
- Use a fixation time of 14–24 hours (longer fixation times 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 digest).

Starting material for DNA purification should be freshly cut sections of FFPE tissue, each with a thickness of up to 10 µm. Up to 8 sections, each with a thickness of up to 10 µm and a surface area of up to 250 mm^2 , 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 3 sections per preparation. Depending on DNA yield and purity, it may be possible to use up to 8 sections in subsequent preparations.

Copurification of RNA

Using the QIAamp DNA FFPE Tissue Kit, RNA may be copurified with the DNA if it is present in the sample. 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, as indicated in the protocol. The protocol describes the use of a 100 mg/ml RNase A stock solution.

For efficient purification of RNA from FFPE tissues, we recommend using the RNeasy® FFPE Kit, which is optimized for high yields of usable RNA from these samples. See ordering information, starting on page 23.

Eluting pure DNA

For downstream applications that require small starting volumes (e.g., some PCR assays), a more concentrated eluate may increase assay sensitivity. QIAamp MinElute columns allow a minimum elution volume of 20 µl for concentrated nucleic acid eluates.

For downstream applications that require a larger starting volume, the elution volume can be increased to 100 µl. However, an increase in elution volume will decrease the concentration of DNA in the eluate.

The volume of eluate recovered may be up to 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.

Buffer ATE should be equilibrated to room temperature (15–25°C) before it is applied to the QIAamp MinElute column. Yields will be increased if the column is incubated with Buffer ATE at room temperature for 5 minutes before centrifugation.

Eluted DNA can be collected in standard 1.5 ml microcentrifuge tubes (not provided). If the purified DNA is to be stored for up to 24 hours, we recommend storage at 2–8°C. For periods longer than 24 hours, we recommend storage at –30°C to –15°C.

For whole genome amplification (WGA) of DNA purified from FFPE tissues, we recommend using the REPLI-g® FFPE Kit, which is optimized for use with this DNA. See ordering information, starting on page 23.

Handling of QIAamp MinElute columns

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

- Carefully apply the sample or solution to the QIAamp MinElute column. Pipet 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.
- Avoid touching the QIAamp MinElute column membrane with the pipet tip.
- After all pulse-vortexing 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.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately

Centrifugation

QIAamp MinElute columns will fit into most standard 1.5–2 ml microcentrifuge tubes. Additional 2 ml collection tubes are available separately.

Centrifugation of QIAamp MinElute columns is performed at 6000 x *g* (8000 rpm) 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 2 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)

Processing QIAamp MinElute columns in a microcentrifuge

- Always close QIAamp MinElute columns before placing them in the microcentrifuge. Centrifuge as described in the protocol.
- 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 collection tubes into which QIAamp MinElute columns can be transferred after centrifugation. Used collection tubes containing flow-through can be discarded, and the new collection tubes containing the QIAamp MinElute columns can be placed directly in the microcentrifuge.

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 Buffer AW1 concentrate. Tick 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.

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 Buffer AW2 concentrate. Reconstituted Buffer AW2 can be stored at room temperature (15–25°C) for up to 1 year.

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

Protocol: Isolation of Genomic DNA from FFPE Tissue Sections

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Read “Important Notes”, page 11.

Things to do before starting

- Equilibrate all buffers to room temperature (15–25°C).
- 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 contain precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffer AW1 and Buffer AW2 have been prepared according to the instructions on page 15.

Procedure

1. Using a scalpel, trim excess paraffin off the sample block.
2. Cut up to 8 sections 5–10 µm thick (see “Starting material”).

   If the sample surface has been exposed to air, discard the first 2–3 sections,
3. Immediately place the sections in a 1.5 or 2 ml microcentrifuge tube (not supplied), and add 1 ml xylene to the sample. Close the lid and vortex vigorously for 10 s.
4. Centrifuge at full speed for 2 min at room temperature (15–25°C).
5. Remove the supernatant by pipetting. Do not remove any of the pellet.

6. Add 1 ml ethanol (96–100%) to the pellet, and mix by vortexing.

   The ethanol extracts residual xylene from the sample.

7. Centrifuge at full speed for 2 min at room temperature.
8. Remove the supernatant by pipetting. Do not remove any of the pellet.

   Carefully remove any residual ethanol using a fine pipet tip.

9. Open the tube and incubate at room temperature or up to 37°C. Incubate for 10 min or until all residual ethanol has evaporated.
10. Resuspend the pellet in 180 µl Buffer ATL. Add 20 µl proteinase K, and mix by vortexing.
11. Incubate at 56°C for 1 h (or until the sample has been completely lysed).
12. Incubate at 90°C for 1 h.

    The incubation at 90°C in Buffer ATL partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA. 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.

13. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.

    If RNA-free genomic DNA is required, add 2 µl RNase A (100 mg/ml) and incubate for 2 min at room temperature before continuing with step 14. Allow the sample to cool to room temperature before adding RNase A.

14. 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.

15. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.

16. Carefully transfer the entire lysate to the QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection 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.

17. Carefully open the QIAamp MinElute column and add 500 µl Buffer AW1 without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

18. Carefully open the QIAamp MinElute column and add 500 µl Buffer AW2 without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

    Contact between the QIAamp MinElute column and the flow-through should be avoided. 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 collection tube from the rotor, so that flow-through does not come into contact with the QIAamp MinElute column.

19. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min to dry the membrane completely.

    This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.

20. Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute column and apply 20–100 µl Buffer ATE to the center of the membrane.

    Important: Ensure that Buffer ATE is equilibrated to room temperature. 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 up to 5 µl less than the volume of elution solution applied to the column.

21. Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.

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

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 protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit **www.qiagen.com**).

		Comments and suggestions
Little or no DNA in the eluate		
a)	Insufficient sample lysis	Proteinase K was stored at high temperatures for a prolonged time. Repeat the procedure using new samples and fresh proteinase K. Make sure that the samples were thoroughly dehydrated prior to embedding. Residual formalin can inhibit the proteinase K digest.
b)	Low-percentage ethanol used instead of 96–100% ethanol	Repeat the purification procedure with new samples using 96–100% ethanol. Do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone.
c)	Buffer AW1 or Buffer AW2 prepared incorrectly	Make sure that the Buffer AW1 and Buffer AW2 concentrates were diluted with the correct volume of 96–100% ethanol, as described on page 15.
Alternative lysis methods		
a)	DNA fragmented or blocked due to formaldehyde modification	Although the 90°C incubation in the QIAamp DNA FFPE Tissue procedure removes most of the formaldehyde modifications, DNA purified from FFPE sections may not perform as well in enzymatic reactions as DNA from fresh or frozen samples. We recommend keeping amplicons as short as possible for PCR (<500 nucleotides).
b)	Reduced sensitivity	Determine the maximum volume of eluate suitable for your amplification reaction. Adjust the volume of eluate added to the amplification reaction accordingly. The elution volume can be adjusted proportionally.
c)	Wash buffers not mixed well	Salt and ethanol components of wash Buffers AW1 and AW2 may have separated out after being unused for a long period. Always mix buffers thoroughly before each purification procedure.

		Comments and suggestions
d)	Ethanol carryover	Be sure to centrifuge at full speed using a new collection tube to completely dry the membrane before elution of DNA.
General handling		
	Clogged QIAamp MinElute column	Incomplete lysis caused clogging of the membrane. Increase the lysis time to fully lyse the sample

Appendix: Working with DNA

General handling

Proper microbiological aseptic technique should always be used when working with small sample sizes. Hands and dust particles may carry bacteria and molds and are the most common sources of contamination. 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. Change gloves frequently and keep tubes closed.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the purification procedure. These tubes are generally DNase-free.

Ordering Information

Product	Contents	Cat. no.
QIAamp DNA FFPE Tissue Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404
Accessories		
RNase A (17,500 U)	2.5 ml (100 mg/ml; 7000 units/ml, solution)	19101
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer	19076
Buffer AL (216 ml)	216 ml Lysis Buffer AL	19075
Buffer AW1 concentrate, 242 ml)	242 ml Wash Buffer (1) Concentrate	19081
Buffer AW2 concentrate, 324 ml)	324 ml Wash Buffer (2) Concentrate	19072
Collection Tubes (2 ml)	1000 Collection Tubes (2 ml)	19201
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect*	Instrument, connectivity package, 1 year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395

Product	Contents	Cat. no.
Related products		
RNeasy FFPE Kit — for purification of total RNA from formalin-fixed, paraffin-embedded tissue sections		
RNeasy FFPE Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, DNase Booster Buffer, RNase-Free Buffers, RNase-Free Water	73504
REPLI-g FFPE Kit — for whole genome amplification of DNA from FFPE tissues		
REPLI-g FFPE Kit (25)*	DNA Polymerase, Buffers and Reagents for 25 x 50 µl whole genome amplification reactions	150243

* All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

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.

Document Revision History

Date	Changes
February 2020	Updated text, ordering information and intended use for QIAcube Connect.

Limited License Agreement for QIAamp DNA FFPE Tissue Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

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Notes

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