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May 2020

# QIAamp<sup>®</sup> DNA FFPE Advanced Handbook

For the isolation of genomic DNA from formalin-fixed, paraffin-embedded (FFPE) tissues using the QIAamp DNA FFPE Advanced or QIAamp DNA FFPE Advanced UNG kits.

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# Kit Contents

The QIAamp DNA FFPE Advanced UNG Kit (cat. no. 56704) consists of 2 boxes, the first one contains the QIAamp DNA FFPE Advanced Kit (cat. no. 56604) and the second box contains Uracil-N-Glycosylase (cat. no. 19160).

<b>Kit</b>	<b>QIAamp DNA FFPE Advanced Kit (50) 56604 50</b>	<b>QIAamp DNA FFPE Advanced UNG Kit (50) 56704 50</b>
Uracil-N-Glycosylase		2 x 1 ml
QIAamp UCP MinElute® Columns	50	50
Collection Tubes (2 ml)	1 x 50	1 x 50
Deparaffinization Solution	2 x 8 ml	2 x 8 ml
Buffer FTB	2 x 0.8 ml	2 x 0.8 ml
Buffer AL*	33 ml	33 ml
Proteinase K	2 x 1.25 ml	2 x 1.25 ml
Buffer AW1*† (concentrate)	19 ml	19 ml
Buffer AW2†† (concentrate)	13 ml	13 ml
RNase-Free Water	2 x 7 ml	2 x 7 ml
RNase A (100mg/ml)	14 mg	14 mg
Buffer ATE‡	12ml	12ml
Quick-Start Protocol	1	1

\* Contains chaotropic salt. Not compatible with disinfecting agents containing bleach; see page 5 for Safety Information.

† Contains sodium azide as a preservative.

†† Before using for the first time, add Ethanol (96-100%) as indicated on the bottle to obtain a working solution.

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## Storage

QIAamp DNA FFPE Advanced UNG Kit (cat. no. 56704) consists of QIAamp DNA FFPE Advanced Kit (cat. no. 56604) and Uracil-N-Glycosylase (cat. no. 19160, UNG). UNG is shipped on dry ice and should be stored immediately upon receipt at  $-30$  to  $-15^{\circ}\text{C}$  in a constant-temperature freezer. Under these conditions, UNG is stable until the expiration date printed on the UNG tube label.

QIAamp UCP MinElute columns should be stored at  $2$ – $8^{\circ}\text{C}$  upon arrival. However, short-term storage (up to 4 weeks) at room temperature ( $15$ – $25^{\circ}\text{C}$ ) does not affect performance. All other components of the QIAamp DNA FFPE Advanced Kit and QIAamp DNA FFPE Advanced UNG Kit should be stored dry at room temperature. Under these conditions, they are stable for at least 12 months.

The QIAamp DNA FFPE Advanced UNG Kit and the QIAamp DNA FFPE Advanced 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 1 year or if ambient temperatures often exceed  $25^{\circ}\text{C}$ , we suggest storing proteinase K at  $2$ – $8^{\circ}\text{C}$ .

## Intended Use

All QIAamp products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

QIAcube<sup>®</sup> 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](http://www.qiagen.com/safety) where you can find, view, and print the SDS for each QIAGEN kit and kit component.

<p>CAUTION</p> 	<p><b>DO NOT add bleach or acidic solutions directly to the sample preparation waste.</b></p>
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Buffer AL and Buffer AW1 contain chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a 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, the components of the QIAamp DNA FFPE Advanced UNG and QIAamp DNA FFPE Advanced Kits are tested against predetermined specifications to ensure consistent product quality.

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# Introduction

Formalin fixation followed by paraffin embedding of tissue specimens is a standard method for the preservation of histological structures of tissues. In addition, the resulting formalin-fixed, paraffin-embedded (FFPE) tissue samples are valuable for molecular analyses. However, DNA preparation from FFPE tissue is associated with several challenges. Yields are often low due to the limited availability of input material and the compromised status of the DNA resulting from fixation and long-term storage. In particular, the recovery of amplifiable DNA strongly depends on removal of formalin-induced cross-links. The QIAamp DNA FFPE Advanced Kits include multiple steps to lyse fixed tissue and remove DNA cross-links.

Additionally, DNA sequence artifacts may be introduced by fixation, embedding, and long-term storage. The most common of these artifacts in FFPE tissues is the deamination of cytosine bases to uracil. This leads to a C-T conversion during amplification, and a false result in downstream analysis. Though present at low frequencies, these artifacts can be critical when using very sensitive methods for mutational analyses such as Next-Generation Sequencing (NGS) with limited starting material. The QIAamp DNA FFPE Advanced UNG procedure includes designated steps to remove deaminated cytosine bases in order to prevent these false results in DNA sequencing analyses.

The QIAamp DNA FFPE Advanced UNG Kit and the QIAamp DNA FFPE Advanced Kit provide convenient, streamlined procedures for efficient purification of high amounts of amplifiable DNA from even difficult to lyse FFPE tissue sections.

## Principle and procedure

The QIAamp DNA FFPE Advanced UNG and the QIAamp DNA FFPE Advanced procedures remove paraffin without the use of xylene or similar solvents and without the need to trim off excess paraffin from the FFPE block in advance. Formalin-induced cross-links are efficiently removed from the DNA prior to purification with the QIAamp UCP MinElute column. Two steps of proteinase K digestion, one before and one after DNA de-crosslinking, ensure complete lysis of even difficult-to-lyse tissue, and facilitate the recovery of high amounts of amplifiable DNA.

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After the initial proteinase K digestion and incubation at elevated temperature to remove cross-links, dilution of the reaction mixture provides conditions that allow the specific removal of deaminated cytosine residues by the enzyme Uracil-N-Glycosylase (UNG). After RNase A digestion and prior to DNA binding, a second proteinase K digestion step improves lysis efficiency and increases yields, particularly for difficult to lyse samples. After the binding of DNA to the QIAamp UCP spin column, residual contaminants are washed away by Buffers AW1 and AW2 and ethanol. Any residual ethanol which may interfere with subsequent enzymatic reactions is removed by an additional centrifugation step. DNA is eluted in 20-100  $\mu$ l to generate highly concentrated DNA. The isolated DNA is compatible with PCR, digital PCR, and next-generation sequencing workflows. Alternatively, it can be stored at  $-20^{\circ}\text{C}$ .

## Starting material

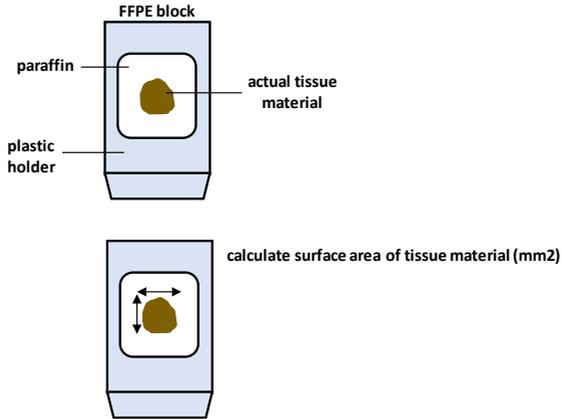
Standard formalin-fixation and paraffin-embedding procedures may cause significant fragmentation of nucleic acids. To limit the extent of nucleic acid fragmentation, be sure to:

- Fix tissue samples in 4%–10% formalin as quickly as possible after surgical removal.
- Use a fixation time of 14–24 h (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 proteinase K digestion).

Sample material for DNA extraction from formalin-fixed, paraffin-embedded (FFPE) tissue is prepared as 5 to 10  $\mu$ m sections cut from a FFPE block using a microtome.

The amount of starting material specified for use with the QIAamp DNA FFPE Advanced UNG Kit and QIAamp DNA FFPE Advanced Kit refers to the actual tissue material of the FFPE sample, excluding the area of paraffin. The starting material is calculated from the surface area of the tissue, the number of sections, and the thickness of sections. With the QIAamp DNA FFPE Advanced UNG Kit and QIAamp DNA FFPE Advanced Kit, FFPE tissue sections of 5–10  $\mu$ m thickness can be processed, totaling up to 4  $\text{mm}^3$  of tissue. In cases where calculating the exact amount is impossible, use no more than 2 sections of 5–10  $\mu$ m thickness.

Sample calculation:



Surface area	No. of sections	Total volume
50 mm <sup>2</sup>	1 section of 10 µm thickness	0.5 mm <sup>3</sup>
	2 sections of 10 µm thickness	1 mm <sup>3</sup>
	4 sections of 10 µm thickness	2 mm <sup>3</sup>
	8 sections of 10 µm thickness	4mm <sup>3</sup>
100 mm <sup>2</sup>	1 section of 10 µm thickness	1 mm <sup>3</sup>
	2 sections of 10 µm thickness	2 mm <sup>3</sup>
	4 sections of 10 µm thickness	4 mm <sup>3</sup>
200 mm <sup>2</sup>	1 section of 10 µm thickness	2 mm <sup>3</sup>
	2 sections of 10 µm thickness	4 mm <sup>3</sup>
400 mm <sup>2</sup>	1 section of 10 µm thickness	4 mm <sup>3</sup>

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## DNA quality and yield

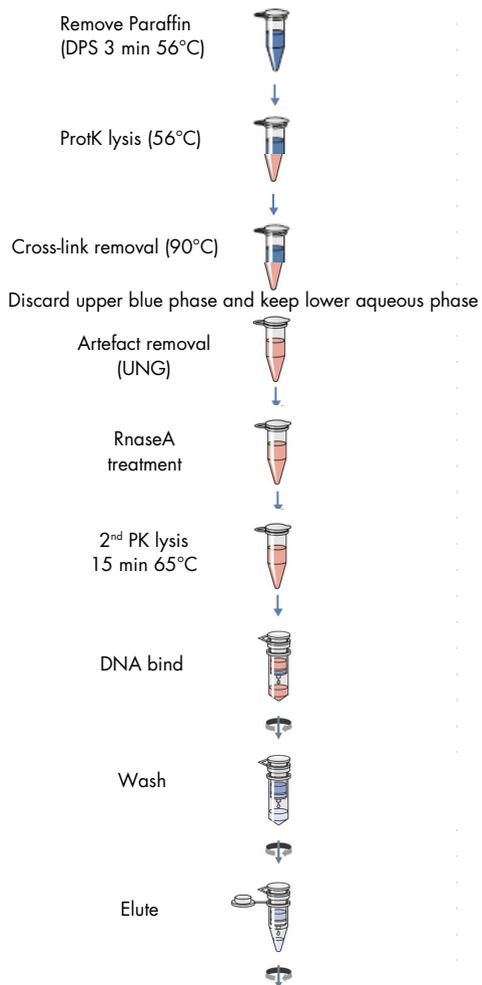
Formalin-fixed, paraffin-embedded tissue material presents challenges not only for the DNA extraction method itself but also for the determination of DNA quality and quantity. Generally, DNA yield from FFPE samples varies greatly, depending on the tissue type, as well as fixation and embedding conditions.

Furthermore, due to the compromised status of the DNA, determination of yield might vary between different quantification methods. While UV-Vis-based measurements will show high absorptions at A260, especially for DNA from samples with heavy fragmentation, fluorometric methodologies using dyes specific for dsDNA (e.g. Qubit) might by contrast show significantly lower DNA recovery. In addition, yield and PCR performance do not necessarily correlate; high yields of DNA as determined by either of the above-mentioned methods might not show good PCR performance. This could be due to the quality of the FFPE sample with regard to DNA fragmentation status and/or the efficiency of cross-link reversal prior to DNA extraction. DNA of a more fragmented status shows far better PCR performance for short amplicons in PCR (<100bp) than DNA of higher molecular weight. However, highly fragmented DNA will not be suitable for PCR applications with amplicons larger than the size of the extracted DNA fragments. If de-crosslinking during DNA purification is insufficient, the extracted DNA will not be properly accessible despite sufficient integrity and poses a poor template for amplification of both small and large fragments in PCR. Thus, DNA yield measured by PCR may differ between large amplicon and short amplicon PCR systems and might also deviate from values obtained by UV-Vis based or fluorometric quantification technologies.

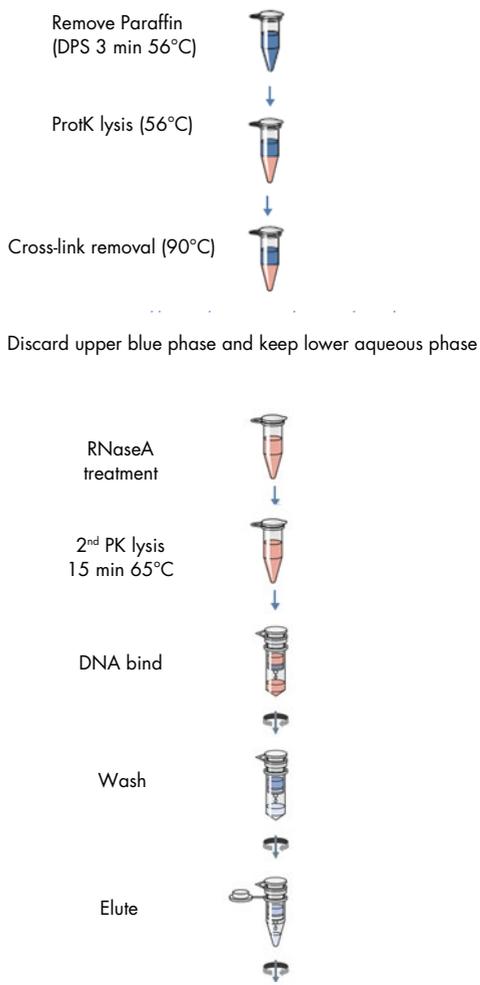
It is recommended to use more than one quality control measure to evaluate DNA quality and quantity, focusing on which downstream application the DNA is intended to be used in. The QIAamp DNA FFPE Advanced UNG Kit and the QIAamp DNA FFPE Advanced Kit provide an optimized workflow for extraction of DNA for use in PCR, digital PCR, and next-generation sequencing analysis using targeted DNA panels or exome sequencing.

# Workflow

## QIAamp DNA FFPE Advanced UNG Kit



## QIAamp DNA FFPE Advanced Kit



# 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 Advanced UNG Kit and the QIAamp DNA FFPE Advanced 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](http://www.qiagen.com/qiacubeprotocols).



**QIAcube Connect.**

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## 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.

- Microcentrifuge with rotor for 2 ml tubes (up to 21,000  $\times g$ )
- Pipettors (2–1000  $\mu\text{l}$ )
- Thermomixer capable of incubation at 90°C
- 96–100% Ethanol (Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.)
- Elution Tubes (e.g. Safe-Lock Tubes (Eppendorf®, cat. no. 0030 120.086) or 1.5 mL SafeSeal microcentrifuge tubes (Sarstedt®, cat no. 72.706)

# Protocol: QIAamp DNA FFPE Advanced UNG Kit

## Important notes before starting

- Deparaffinization Solution solidifies at temperatures below 18°C. Incubate at 30°C to redissolve.
- If Buffer AL contains precipitate, dissolve by heating at 70°C with gentle agitation.
- Add 25 ml ethanol (96–100%) to the bottle containing 19 ml of 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 for up to 1 year.

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

- Add 30 ml ethanol (96–100%) to the bottle containing 13 ml of Buffer AW2 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW2 can be stored at room temperature for up to 1 year.

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

- Preheat a thermomixer at 56°C for use in steps 2 and 4, and preheat a second thermomixer at 90°C for use in step 5.

## Procedure

1. Place the FFPE sections in a 1.5 ml or 2 ml microcentrifuge tube (not supplied). Add 300 µl Deparaffinization Solution, vortex vigorously for 10 s, and centrifuge briefly to bring the sample to the bottom of the tube.
2. Incubate at 56°C for 3 min, then allow to cool to room temperature.

**Note:** If too little Deparaffinization Solution is used or if larger amounts of paraffin are carried over with the sample, Deparaffinization Solution may become waxy or solid after

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cooling. If this occurs, add additional Deparaffinization Solution and repeat the incubation at 56°C.

3. Add 25 µl Buffer FTB, 55 µl RNase-free Water, and 20 µl Proteinase K. Mix by vortexing. Briefly centrifuge the tube to spin down any FFPE tissue that sticks to the tube wall or under the cap of the tube after vortexing.

**Note:** A master mix that comprises the respective components may be prepared in advance.

4. Incubate for 1 h at 56°C and 1000 rpm.

**Note:** After incubation, set the thermomixer to 50°C for incubation in step 6.

5. Incubate for 1 h at 90°C without shaking.

**Optional:** After incubation, briefly centrifuge the tube to remove drops from inside the lid.

6. Carefully remove and discard the upper blue phase. Keep the lower aqueous lysate, and add 115 µl RNase-free Water and 35 µl UNG. Vortex and incubate at 50°C for 5 min without shaking.

**Optional:** After incubation, briefly centrifuge the tube to remove drops from inside the lid.

**Note:** Transfer of small volumes of Deparaffinization Solution (upper blue phase) will not affect the DNA purification.

**Note:** After incubation, set the thermomixer to 65°C for incubation in step 8.

7. Add 2 µl RNase A, vortex, and incubate for 2 min at room temperature on the bench.

**Optional:** After incubation, briefly centrifuge the tube to remove drops from inside the lid.

8. Add 20 µl Proteinase K, vortex, and incubate for 15 min at 65°C and 450 rpm.

**Optional:** After incubation, briefly centrifuge the tube to remove drops from inside the lid.

9. Add 250 µl Buffer AL and 250 µl ethanol (96–100%) to each sample and mix thoroughly by vortexing.

**Optional:** Centrifuge briefly to remove drops from inside the lid.

10. Transfer 450 µl lysate to the QIAamp UCP MinElute column (in a 2 ml collection tube), and centrifuge at 15000 × *g* for 30 s.

11. Transfer the residual lysate to the same QIAamp UCP MinElute column, and centrifuge at  $15000 \times g$  for 1 min. Discard the flow-through from step 10 and 11 and reuse the collection tube.
12. Add 500  $\mu$ l Buffer AW1 to each spin column, and centrifuge at  $15000 \times g$  for 30 s. Discard the flow-through and reuse the collection tube.
13. Add 500  $\mu$ l Buffer AW2 to each spin column, and centrifuge at  $15000 \times g$  for 30 s. Discard the flow-through and reuse the collection tube.
14. Add 250  $\mu$ l ethanol (96–100%) to the spin column, and centrifuge at  $15000 \times g$  for 30 s. Discard the flow-through and collection tube. Place the spin column into a new 2 ml collection tube (supplied) and centrifuge for 3 min at full speed to remove any residual liquid to dry the membrane
15. Place the QIAamp UCP MinElute column into a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the flow-through. Open the lid of the QIAamp MinElute column and apply 20–100  $\mu$ l Buffer ATE to the center of the membrane.
16. Close the lid and incubate at room temperature for 1 min, then centrifuge at full speed for 1 min to elute the DNA.

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

When using small elution volumes, DNA yield can be increased by re-applying the first eluate to the spin column membrane, then incubate for 3 min at room temperature and centrifuge again for 1 min at full speed.

# Protocol: QIAamp DNA FFPE Advanced Kit

## Important notes before starting

- Deparaffinization Solution solidifies at temperatures below 18°C. Incubate at 30°C to redissolve.
- If Buffer AL contains precipitate, dissolve by heating at 70°C with gentle agitation.
- Add 25 ml ethanol (96–100%) to the bottle containing 19 ml of 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 for up to 1 year.  
**Note:** Before starting the procedure, mix reconstituted Buffer AW1 by shaking.
- Add 30 ml ethanol (96–100%) to the bottle containing 13 ml of Buffer AW2 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW2 can be stored at room temperature for up to 1 year.  
**Note:** Before starting the procedure, mix reconstituted Buffer AW2 by shaking.
- Preheat a thermomixer at 56°C for use in steps 2 and 4, and preheat a second thermomixer at 90°C for use in step 5.

## Procedure

1. Place the FFPE sections in a 1.5 ml or 2 ml microcentrifuge tube (not supplied). Add 300 µl Deparaffinization Solution, vortex vigorously for 10 s, and centrifuge briefly to bring the sample to the bottom of the tube.

2. Incubate at 56°C for 3 min, then allow to cool to room temperature.

**Note:** If too little Deparaffinization Solution is used or if larger amounts of paraffin are carried over with the sample, Deparaffinization Solution may become waxy or solid after cooling. If this occurs, add additional Deparaffinization Solution and repeat the incubation at 56°C.

3. Add 25 µl Buffer FTB, 55 µl RNase-free Water, and 20 µl Proteinase K. Mix by vortexing. Briefly centrifuge the tube to spin down any FFPE tissue that sticks to the tube wall or under the cap of the tube after vortexing.

**Note:** A master mix that comprises the respective components may be prepared in advance.

4. Incubate for 1 h at 56°C and 1000 rpm.  
**Note:** After incubation, set the thermomixer to 65°C for incubation in step 8.
5. Incubate for 1 h at 90°C without shaking.  
**Optional:** After incubation, briefly centrifuge the tube to remove drops from inside the lid.
6. Carefully remove and discard the upper blue phase. Keep the lower aqueous lysate, add 150 µl RNase-free Water, then vortex.  
**Optional:** After incubation, briefly centrifuge the tube to remove drops from inside the lid.  
**Note:** Transfer of small volumes of Deparaffinization Solution (upper blue phase) will not affect the DNA purification.  
**Note:** After incubation, set the thermomixer to 65°C for incubation in step 8.
7. Add 2 µl RNase A, vortex, and incubate for 2 min at room temperature on the bench.  
**Optional:** After incubation, briefly centrifuge the tube to remove drops from inside the lid.
8. Add 20 µl Proteinase K, vortex, and incubate for 15 min at 65°C and 450 rpm.  
**Optional:** After incubation, briefly centrifuge the tube to remove drops from inside the lid.
9. Add 250 µl Buffer AL and 250 µl ethanol (96–100%) to each sample and mix thoroughly by vortexing.  
**Optional:** Centrifuge briefly to remove drops from inside the lid.
10. Transfer 450 µl lysate to the QIAamp UCP MinElute column (in a 2 ml collection tube), and centrifuge at 15000 x *g* for 30 s.
11. Transfer the residual lysate to the same QIAamp UCP MinElute column, and centrifuge at 15000 x *g* for 1 min. Discard the flow-through from step 10 and 11 and reuse the collection tube.
12. Add 500 µl Buffer AW1 to each spin column, and centrifuge at 15000 x *g* for 30 s. Discard the flow-through and reuse the collection tube.
13. Add 500 µl Buffer AW2 to each spin column, and centrifuge at 15000 x *g* for 30 s. Discard the flow-through and reuse the collection tube.

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14. Add 250  $\mu$ l ethanol (96–100%) to the spin column, and centrifuge at 15000  $\times g$  for 30 s. Discard the flow-through and collection tube. Place the spin column into a new 2 ml collection tube (supplied) and centrifuge for 3 min at full speed to remove any residual liquid to dry the membrane.
  15. Place the QIAamp UCP MinElute column into a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the flow-through. Open the lid of the QIAamp MinElute column and apply 20–100  $\mu$ l Buffer ATE to the center of the membrane.
  16. Close the lid and incubate at room temperature for 1 min, then centrifuge at full speed for 1 min to elute the DNA.

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

When using small elution volumes DNA yield can be increased by re-applying the first eluate to the spin column membrane, then incubate for 3 min at room temperature and centrifuge again for 1 min at full speed.

# 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](http://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, visit [www.qiagen.com](http://www.qiagen.com).

## Comments and suggestions

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### Little or no DNA in the eluate

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|---|---|
| a) Poor quality of starting material                      | Samples that were fixed for over 20 h or stored for very long periods of time may contain very little usable nucleic acids.<br>Sections that were mounted on microscope slides may yield very little usable nucleic acids due to prolonged exposure to air. |
| b) Buffer AW1 or Buffer AW2 prepared incorrectly          | Make sure that Buffer AW1 or Buffer AW2 concentrates were diluted with the correct volume of 96–100% ethanol, as described in “Important notes before starting”.  |
| c) Low percentage ethanol used instead of 96–100% ethanol | Repeat the purification procedure with new samples using 96–100% ethanol. Do not use denatured ethanol.   |
| d) DNA still bound to spin column                         | Repeat the elution step, but incubate the QIAamp UCP MinElute spin column on the benchtop for 10 min with Buffer ATE before centrifuging.   |

### Inefficient removal of deaminated cytosine

- |  |  |
|--|--|
| a) Too much starting material                | Since the QIAamp DNA FFPE Advanced UNG Kit is based on an enzymatic digestion, too much starting material will lead to inefficiency. Reduce the amount of starting material. |
| b) UNG reaction mixture prepared incorrectly | Be sure to properly prepare the reaction mix by precise addition of all components and transfer of the aqueous phase in steps 3 and 6.                                       |

### Clogged QIAamp UCP MinElute spin column

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|------------------------------------|---|
| Centrifugation temperature too low | The centrifugation temperature should be 15–25°C. Some centrifuges may cool to below 15°C even when set at 20°C. This can cause formation of precipitates that can clog the QIAamp MinElute spin column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol-containing sample to 37°C before transferring it to the QIAamp mini spin column. |
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# Ordering Information

Product	Contents	Cat. no.
QIAamp DNA FFPE Advanced UNG Kit (50)	For 50 preps: Uracil-N-Glycosylase, QIAamp UCP MinElute Columns, Collection Tubes, Deparaffinization Solution, Proteinase K, RNase A, RNase-free Water, and Buffers	56704
QIAamp DNA FFPE Advanced Kit (50)	For 50 preps: QIAamp UCP MinElute Columns, Collection Tubes, Deparaffinization Solution, Proteinase K, RNase A, RNase-free Water, and Buffers	56604
Uracil-N-Glycosylase (2 x 1 ml)		19160
<b>Accessories and Reagents</b>		
Deparaffinization Solution (16 ml)	2 x 8 ml Deparaffinization Solution	19093
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
Collection Tubes (2 ml)	1000 Collection Tubes (2 ml)	19201
RNase A (17,500 U)	2.5 ml (100 mg/ml; 7000 units/ml, solution)	19101

Product	Contents	Cat. no.
<b>QIAcube Connect – for fully automated nucleic acid extraction with QIAGEN spin-column kits</b>		
QIAcube Connect*	Instrument, connectivity package, 1-year warranty on parts and labor	Inquire

\* 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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

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## Notes

# Handbook Revision History

Date	Changes
05/2020	Initial release

## Limited License Agreement for QIAamp DNA FFPE Advanced UNG Kit and QIAamp DNA FFPE Advanced 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](http://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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3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
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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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