
June 2020

QIAamp[®] MinElute[®] ccfDNA Handbook

For concentration and purification of
circulating cell-free DNA from plasma
or serum

Contents

Kit Contents	3
Storage	5
Intended Use	5
Safety Information	6
Quality Control	6
Introduction	7
Principle and workflow	7
Sample volumes	8
Sample numbers	8
Procedure	8
Yield and size of nucleic acids	10
Automated purification of DNA on QIAcube instruments	11
Equipment and Reagents to Be Supplied by User	12
Important Notes	13
Preparation of buffers	13
Protocol: Purification of Circulating DNA from 1–4 ml Serum or Plasma Using the QIAamp MinElute ccfDNA Mini Kit	14
Protocol: Purification of Circulating DNA from 4–10 ml Serum or Plasma Using the QIAamp MinElute ccfDNA Midi Kit	17
Troubleshooting Guide	20
Appendix: Recommendations for Plasma Separation and Storage	22
Ordering Information	24
Document Revision History	26

Kit Contents

QIAamp MinElute ccfDNA Mini Kit (50)	(50)
Catalog no.	55204
Number of preps	50
QIAamp UCP MinElute Columns	50
Collection Tubes 2 ml	100
Collection Tubes 1.5 ml	50
Ultra-clean Water	5 x 1.0 ml
Proteinase K	10 ml
Bead Binding Buffer	20 ml
Magnetic Bead Suspension	2 x 1.6 ml
Bead Elution Buffer	12 ml
Buffer ACB	12 ml
Buffer ACW2	13 ml
Bead Elution Tubes	100
Quick-Start Protocol	1

QIAamp MinElute ccfDNA Midi Kit (50)	(50)
Catalog no.	55284
Number of preps	50
QIAamp UCP MinElute Columns	50
Collection Tubes 2 ml	100
Collection Tubes 1.5 ml	50
Ultra-clean Water	5 x 1.0 ml
Proteinase K	2 x 10 ml
Bead Binding Buffer	2 x 20 ml
Magnetic Bead Suspension	5 x 1.6 ml
Bead Elution Buffer	12 ml
Buffer ACB	12 ml
Buffer ACW2	13 ml
Bead Elution Tubes	100
Quick-Start Protocol	1

Storage

QIAamp UCP MinElute columns and Magnetic Bead Suspension should be stored at 2–8°C upon arrival.

QIAamp MinElute ccfDNA Kits contain a ready-to-use proteinase K solution, which is dissolved in a specially formulated storage buffer. When stored at room temperature (15–25°C), the proteinase K is stable for up to 1 year after delivery, unless otherwise stated on the label. To prolong the lifetime of proteinase K, storage at 2–8°C is recommended.

Intended Use

QIAamp MinElute ccfDNA Mini and Midi Kits are for molecular biology applications. These products are 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 these 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.

<p>CAUTION</p> 	<p>CAUTION: DO NOT add bleach or acidic solutions directly to waste containing Buffer ACB.</p>
---	--

Buffer ACB contains guanidine salts, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially 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 MinElute ccfDNA Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

Free-circulating DNA, such as tumor-specific extracellular DNA fragments in the blood or fetal DNA in maternal blood, are present in serum or plasma, usually as short fragments of <1000 bp.

QIAamp MinElute ccfDNA Kits enable efficient purification of these circulating DNAs from human plasma or serum. Samples can be either fresh or frozen (provided that they have not been frozen and thawed more than once) or stabilized in a solution, such as in the PAXgene® Blood ccfDNA Tube. Preconcentration of ccfDNA on magnetic beads allows flexible and scalable handling of volumes from 1 to 10 ml, while the use of a MinElute column allows flexible elution volumes between 20 and 80 µl, providing the highest possible concentration of nucleic acid species that are present in low concentrations (typically 1–100 ng/ml circulating DNA in human plasma).

Free-circulating DNA is eluted in Ultra-clean Water, ready for use in downstream reactions or for storage at –30 to –15°C. Purified nucleic acids are free of proteins, nucleases, and other impurities.

Principle and workflow

QIAamp MinElute ccfDNA Kits use a fast procedure involving preconcentration of circulating nucleic acids onto magnetic beads and a cleanup of the resulting preeluate on QIAamp UCP MinElute columns in a quick and convenient spin procedure. The robust procedure helps to eliminate sample-to-sample cross-contamination and increases user safety when handling potentially infectious samples.

The simple procedure, which is highly suited for simultaneous processing of multiple samples, provides pure nucleic acids in less than 1 hour for 24 samples.

Sample volumes

The QIAamp MinElute ccfDNA Mini Kit has been optimized for sample volumes from 1 to 4 ml, while the QIAamp MinElute Midi Kit has been optimized for sample volumes from 4 to 10 ml. QIAamp UCP MinElute columns can bind fragmented nucleic acids that are as short as 20 bases, but yield depends on the sample volume and the concentration of circulating nucleic acids in the sample (typically 1–100 ng/ml in plasma).

Sample numbers

QIAamp MinElute ccfDNA Kits have been designed to be flexible with regard to plasma input volumes, and the bead-binding procedure to generate the preeluate can be scaled as desired. Each kit has been designed with a specific target volume in mind: the Mini kit is designed to process fifty 1–2 ml plasma samples, while the Midi kit has been designed to process fifty 4–5 ml plasma samples. Processing larger volumes is possible with these kits, but doing so will directly reduce the number of preparations that can be performed in total. Thus, the Mini kit can be scaled to 4 ml plasma samples, but then the kit will contain only enough bead-binding and elution reagents for 25 samples in total. The Midi kit can be scaled to 10 ml plasma samples, but then it will only have sufficient reagents to process 25 samples. By designing the kits to adapt to the most commonly required scenarios, we are able to keep prices low and minimize waste. If you need to regularly process volumes of 10 ml or higher, please contact QIAGEN Technical Services for advice.

Procedure

Preparing plasma from whole blood

To isolate circulating cell-free nucleic acids from blood samples, we recommend following the protocol described in “Appendix: Recommendations for Plasma Separation and Storage”, page 22, which includes a high *g*-force centrifugation step to remove cellular debris and thereby reduce the amount of cellular or genomic DNA and RNA in the sample. Whole blood

must be stabilized to separate circulating nucleic acids from genomic DNA. Appropriate tubes are the PAXgene Blood ccfDNA Tube, BD Vacutainer® (or other primary blood tubes) containing EDTA as an anti-coagulant, or Streck® Cell-Free DNA BCT.

Lysing samples and binding to beads

Up to 10 ml of plasma or serum can be processed in a standard 15 ml centrifuge tube. Bead Binding Buffer, Magnetic Bead Suspension, and Proteinase K are added to the sample in the appropriate ratio to the sample volume. Circulating DNA is bound to magnetic beads during end-over-end rotation, during which the samples are lysed at room temperature in the presence of proteinase K, which ensures complete release of nucleic acids from bound proteins.

Bead collection and elution

Magnetic beads with bound DNA are collected in a pellet on a magnet rack, and the supernatant is discarded. The bound DNA is then eluted from the beads in a special buffer, after which the beads (now with no DNA bound to them) are separated from the preeluate containing the DNA.

Adsorption to the QIAamp MinElute membrane

Buffer ACB is added to the preeluate to adjust conditions to allow optimal binding of the circulating nucleic acids to the membrane. Preeluates are then transferred onto a QIAamp UCP MinElute column, and circulating DNA is adsorbed onto the silica membrane as the mixture is spun through in a centrifuge. Salt and pH conditions ensure that proteins and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not retained on the QIAamp MinElute membrane.

Removal of residual contaminants

Nucleic acids remain bound to the membrane, while contaminants are efficiently washed away in a single wash step. The membrane is then dried to ensure that no residual ethanol is carried over into the elution step.

Elution of pure nucleic acids

Elution is performed using Ultra-clean Water. The elution volume can be as low as 20 μl for highly concentrated nucleic acids.

For downstream applications that require small starting volumes (e.g., some PCR and RT-PCR assays), a more concentrated eluate may increase assay sensitivity.

For downstream applications that require a larger starting volume, the elution volume can be increased up to 80 μl . However, an increase in elution volume will decrease the concentration of nucleic acids in the eluate.

The eluate volume recovered can be up to 5 μl less than the volume of elution buffer applied to the column; for example, an elution volume of 20 μl results in >15 μl final eluate. The volume of recovered eluate depends on the nature of the sample.

Eluted DNA is collected in 1.5 ml microcentrifuge tubes. If the purified circulating nucleic acids are to be stored for up to 24 hours, storage at 2–8°C is recommended. For periods of storage longer than 24 hours, storage at –30 to –15°C is recommended.

Yield and size of nucleic acids

Yields of free-circulating nucleic acids isolated from biological samples are normally well below 1 μg and are therefore difficult to determine with a spectrophotometer. Additionally the small size of circulating DNA (main peak, approximately 160–180 bp) renders many fluorometric methods unreliable for yield determination. Quantitative amplification methods, such as real-time PCR, are recommended for determination of yields.

The size distribution of circulating nucleic acids purified using this procedure can be checked by analysis on an Agilent Bioanalyzer®, TapeStation® or similar device. Alternatively, agarose gel electrophoresis and hybridization to a target-specific labeled probe is an option (Sambrook, J. and Russell, D.W. [2001] *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Automated purification of DNA on QIAcube instruments

Purification of DNA, including ccfDNA, 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 MinElute ccfDNA Kit for purification of high-quality ccfDNA.

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.

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.

In addition to the QIAamp MinElute ccfDNA Mini or Midi Kit, the following supplies are required:

Equipment

- Microcentrifuge
- Shaker for microcentrifuge tubes, for example, the Thermomixer® Comfort (Eppendorf cat. no. 5355 000.011) with appropriate block
- End-over-end shaker/rotator
- Magnet rack for 15 ml tubes (15 ml/50 ml Tube Magnet, cat. no. 36935; AdnaMag-L, cat. no. 399921; or equivalent)
- Magnet rack for 2 ml tubes (12-Tube Magnet, cat. no. 36912; AdnaMag-S, cat. no. 399911; or equivalent)

Material

- Pipettes (adjustable)
- Sterile pipette tips (pipette tips with aerosol barriers are recommended to help prevent cross-contamination)
- 15 ml centrifuge tubes

Reagents

- Ethanol (96–100%)
- Isopropanol (100%)

Important Notes

Preparation of buffers

Buffer ACB

Before use, add 8 ml isopropanol (100%) to 12 ml Buffer ACB concentrate to obtain 20 ml Buffer ACB. Mix well after adding isopropanol.

Buffer ACW2

Before use, add 30 ml ethanol (96–100%) to 13 ml Buffer ACW2 concentrate to obtain 43 ml Buffer ACW2. Mix well after adding ethanol.

Protocol: Purification of Circulating DNA from 1–4 ml Serum or Plasma Using the QIAamp MinElute ccfDNA Mini Kit

Things to do before starting

- Add isopropanol to Buffer ACB concentrate (see “Preparation of buffers”, page 13).
- Add ethanol to Buffer ACW2 concentrates (see “Preparation of buffers”, page 13).
- Prepare a shaker for microcentrifuge tubes at room temperature for use in step 4.
- Preheat a second shaker at 56°C for use in step 10. (Alternatively, equilibrate the first shaker to 56°C after step 4.)
- Resuspend Magnetic Bead Suspension by pulse-vortexing for 1 min. Do not let the suspension settle for more than 2 min. Pipet from the center of the suspension.

Procedure

1. Mix components according to Table 1 in a 15 ml tube (not provided), and incubate for 10 min at room temperature while shaking (slow speed) end-over-end.

Table 1. Component mix (QIAamp MinElute ccfDNA Mini Kit)

Plasma (ml)	Magnetic Bead Suspension (µl)	Proteinase K (µl)	Bead Binding Buffer (µl)
1	30	55	150
2	60	110	300
3	90	165	450
4	120	220	600

2. Spin briefly (30 s at 200 x *g*) to remove any solution in cap.
3. Place the tube containing bead solution into a magnetic rack for 15 ml tubes. Let stand for at least 1 min, until the solution is clear. Discard supernatant.
4. Remove the tube from the magnetic rack and add 200 μ l Bead Elution Buffer to the bead pellet. Vortex to resuspend beads, and pipet up and down to mix and rinse residual beads from the tube wall. Transfer the mixture (including beads) into a Bead Elution Tube. Incubate for 5 min on a shaker for microcentrifuge tubes at room temperature and 300 rpm.

Note: If the same shaker for microcentrifuge tubes is to be used in step 10, remove the tubes after the room temperature incubation and equilibrate the shaker to 56°C.

5. Place the Bead Elution Tube containing the bead solution into a magnetic rack for 2 ml tubes. Let stand for at least 1 min, until the solution is clear.
6. Transfer the supernatant into a new Bead Elution Tube. Add 300 μ l Buffer ACB, and vortex to mix. Briefly centrifuge the tube to remove drops from inside the lid. Discard the bead pellet.

Note: Avoid transferring any magnetic beads in this step. Carryover may result in reduced ccfDNA yield.

7. Pipet the supernatant–Buffer ACB mixture from step 6 into a QIAamp UCP MinElute column, and centrifuge for 1 min at 6000 x *g*. Place the QIAamp UCP MinElute column into a clean 2 ml collection tube, and discard the flow-through.
8. Add 500 μ l Buffer ACW2 to the QIAamp UCP MinElute Column, and centrifuge for 1 min at 6000 x *g*. Place the QIAamp UCP MinElute column into a clean 2 ml collection tube, and discard the flow-through.
9. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.

-
10. Place the QIAamp UCP MinElute column into a clean 1.5 ml elution tube (provided), and discard the 2 ml collection tube from step 9. Open the lid, and incubate the assembly in a shaker for microcentrifuge tubes at 56°C for 3 min to dry the membrane completely.
 11. Carefully pipet 20–80 μ l of Ultra-clean Water onto the center of the membrane. Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 $\times g$; 14,000 rpm) for 1 min to elute the nucleic acids.

Note: To maximize yield eluted in 20–80 μ l, reapply the eluate to the column for re-elution. Place the QIAamp UCP MinElute Column in a clean 1.5 ml elution tube (not provided). Aspirate the eluate in the 1.5 ml elution tube from step 10 and reload it onto the center of the membrane. Close the lid, and incubate 1 min at RT. Centrifuge 1 min at full speed (20,000 $\times g$; 14,000 rpm).

Protocol: Purification of Circulating DNA from 4–10 ml Serum or Plasma Using the QIAamp MinElute ccfDNA Midi Kit

Things to do before starting

- Add isopropanol to Buffer ACB concentrate (see “Preparation of buffers”, page 13).
- Add ethanol to Buffer ACW2 concentrates (see “Preparation of buffers”, page 13).
- Prepare a shaker for microcentrifuge tubes at room temperature for use in step 4.
- Preheat a second shaker at 56°C for use in step 10. (Alternatively, equilibrate the first shaker to 56°C after step 4.)
- Resuspend Magnetic Bead Suspension by pulse-vortexing for 1 min. Do not let the suspension settle for more than 2 min. Pipet from the center of the suspension.

Procedure

1. Mix components according to Table 2 in a 15 ml tube (not provided), and incubate for 10 min at room temperature while shaking (slow speed) end-over-end.

Table 2. Component mix (QIAamp MinElute ccfDNA Midi Kit)

Plasma (ml)	Magnetic Bead Suspension (µl)	Proteinase K (µl)	Bead Binding Buffer (µl)
4	120	220	600
5	150	275	750
6	180	330	900
7	210	385	1050
8	240	440	1200
9	270	495	1350
10	300	550	1500

2. Spin briefly (30 s at 200 x *g*) to remove any solution in the cap.
3. Place the tube containing bead solution into a magnetic rack for 15 ml tubes. Let stand for at least 1 min, until the solution is clear. Discard supernatant.
4. Remove the tube from the magnetic rack and add 200 μ l Bead Elution Buffer to the bead pellet. Vortex to resuspend beads, and pipet up and down to mix and rinse residual beads from the tube wall. Transfer the mixture (including beads) into a Bead Elution Tube. Incubate for 5 min on a shaker for microcentrifuge tubes at room temperature and 300 rpm.

Note: If the same shaker for microcentrifuge tubes is to be used in step 10, remove the tubes after the room temperature incubation and equilibrate the shaker to 56°C.

5. Place the Bead Elution Tube containing the bead solution into a magnetic rack for 2 ml tubes. Let stand for at least 1 min, until the solution is clear.
6. Transfer the supernatant into a new Bead Elution Tube. Add 300 μ l Buffer ACB, and vortex to mix. Briefly centrifuge the tube to remove drops from inside the lid. Discard the bead pellet.

Note: Avoid transferring any magnetic beads in this step. Carryover may result in reduced ccfDNA yield.

7. Pipet the supernatant–Buffer ACB mixture from step 6 into a QIAamp UCP MinElute column, and centrifuge for 1 min at 6000 x *g*. Place the QIAamp UCP MinElute column into a clean 2 ml collection tube, and discard the flow-through.
8. Add 500 μ l Buffer ACW2 to the QIAamp UCP MinElute Column, and centrifuge for 1 min at 6000 x *g*. Place the QIAamp UCP MinElute column into a clean 2 ml collection tube, and discard the flow-through.
9. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.
10. Place the QIAamp UCP MinElute column into a clean 1.5 ml elution tube (provided), and discard the 2 ml collection tube from step 9. Open the lid, and incubate the assembly in a shaker for microcentrifuge tubes at 56°C for 3 min to dry the membrane completely.

11. Carefully pipet 20–80 μ l of Ultra-clean Water into the center of the membrane. Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 \times g ; 14,000 rpm) for 1 min to elute the nucleic acids.

Note: To maximize yield eluted in 20–80 μ l, reapply the eluate to the column for re-elution. Place the QIAamp UCP MinElute Column in a clean 1.5 ml elution tube (not provided). Aspirate the eluate in the 1.5 ml elution tube from step 10 and reload it onto the center of the membrane. Close the lid, and incubate 1 min at RT. Centrifuge 1 min at full speed (20,000 \times g ; 14,000 rpm).

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 in 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 or protocols in this handbook (for contact information, visit support.qiagen.com).

Comments and suggestions

Little or no nucleic acids in the eluate

- | | |
|---|--|
| a) Primary blood tube contains an anticoagulant other than EDTA | Anticoagulants other than EDTA may lead to accelerated DNA degradation compared to EDTA blood. Repeat the purification procedure with new samples. |
| b) Extended time between blood draw and plasma preparation | Blood cells may disintegrate and release genomic DNA into the plasma, diluting the target nucleic acid. |
| c) Samples frozen and thawed more than once | Repeated freezing and thawing should be avoided, because this may lead to DNA degradation. Always use fresh samples or samples that have been thawed only once. |
| d) Low concentration of target DNA in the samples | Samples were left standing at room temperature for too long. Repeat the purification procedure with new samples. |
| e) Low-percentage ethanol used instead of 96–100% | Repeat the purification procedure with new samples and 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. |
| f) Buffer ACB prepared incorrectly | Check that Buffer ACB concentrate was reconstituted with the correct volume of isopropanol (not ethanol, see “Preparation of buffers”, page 13). |
| g) Buffer ACW2 prepared incorrectly | Check that Buffer ACW2 concentrate was diluted with the correct volume of ethanol (see “Preparation of buffers”, page 13). Repeat the purification procedure with new samples. |

Comments and suggestions

- | | |
|--|--|
| h) Buffer ACW2 prepared with 70% ethanol | Check that Buffer ACW2 concentrate was diluted with 96–100% ethanol (see “Preparation of buffers”, page 13). Repeat the purification procedure with new samples. |
| i) Wrong measurement method used | Due to low yields and the small size of ccfDNA fragments, not all measurement methods are reliable; see “Yield and size of nucleic acids”, page 10. |

DNA or RNA do not perform well in downstream enzymatic reactions

- | | |
|--|---|
| a) Little or no DNA in the eluate | See troubleshooting section on “Little or no nucleic acids in the eluate” for possible reasons. Increase the amount of eluate added to the reaction if possible. |
| b) Inappropriate elution volume used | Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly. The elution volume can be adapted proportionally. |
| c) Buffers not mixed thoroughly | Salt and ethanol components of wash Buffer ACW2 may have separated out after being left for a long period between runs. Always mix buffers thoroughly before each run. |
| d) New <i>Taq</i> DNA polymerase or PCR chemistry used | If enzymes are changed, it may be necessary to readjust the amount of eluate used for PCR. |

General handling

- | | |
|--------------------------|--|
| Variable elution volumes | Different samples can affect the volume of the final eluate. The recovered eluate volume will be up to 5 μ l less than the elution volume applied to the QIAamp UCP MinElute column. |
|--------------------------|--|

Appendix: Recommendations for Plasma Separation and Storage

To isolate circulating cell-free nucleic acids from blood samples, we recommend following this protocol, which includes a high *g*-force centrifugation step to remove cellular debris and thereby reduce the amount of cellular or genomic DNA and RNA in the sample. Whole blood must be stabilized to separate circulating nucleic acids from genomic DNA. Appropriate tubes are the PAXgene Blood ccfDNA Tube, BD Vacutainer (or other primary blood tubes) containing EDTA as an anti-coagulant, or Streck Cell-Free DNA BCT.

Procedure

1. Place whole blood in a collection tube into a centrifuge with a swing-out rotor and appropriate buckets.
2. Centrifuge the blood samples for 10 min at 1900 x *g* (3000 rpm) with temperature set to 4°C.
3. Carefully aspirate plasma supernatant without disturbing the buffy coat layer. Approximately 4–5 ml plasma can be obtained from one 10 ml primary blood tube.

Note: Plasma can be used for circulating nucleic acid extraction at this stage. However, the high-speed centrifugation that follows will remove additional cellular debris and contamination of the circulating nucleic acids by genomic DNA and RNA derived from damaged blood cells.

4. Transfer aspirated plasma into new 15 ml centrifuge tubes with conical bottoms.
5. Centrifuge the plasma samples for 10 min at 16,000 x *g* in a fixed-angle rotor with temperature set to 4°C.

This will remove additional cellular nucleic acids attached to cell debris.

6. Using a pipette, carefully transfer the supernatant into a new tube without disturbing the pellet.

-
7. If plasma will be used for nucleic acid extraction on the same day, store at 2–8°C until further processing. For longer storage, keep plasma frozen at –90 to –65°C. Before using the plasma for circulating nucleic acid extraction, thaw plasma tubes at room temperature.
 8. In case of cryoprecipitates, follow these 2 steps:
 - 8a. Centrifuge plasma sample for 5 min at 16,000 x *g* in fixed angle rotor with temperature set to 4°C.
 - 8b. Transfer the supernatant into a new tube, and then begin with the nucleic acid extraction protocol.

Ordering Information

Product	Contents	Cat. no.
QIAamp MinElute ccfDNA Mini Kit (50)	For 50 preps: QIAamp UCP MinElute Columns, QIAGEN Proteinase K, Magnetic Bead Suspension, Buffers, Bead Elution Tubes and Collection Tubes (1.5 ml and 2 ml)	55204
QIAamp MinElute ccfDNA Midi Kit (50)	For 50 preps: QIAamp UCP MinElute Columns, QIAGEN Proteinase K, Magnetic Bead Suspension, Buffers, Bead Elution Tubes and Collection Tubes (1.5 ml and 2 ml)	55284
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	200 µl filter-tips (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395
Related products		
PAXgene Blood ccfDNA Tubes (100)	100 blood collection tubes (10 ml), to be used in conjunction with QIAGEN QIAamp MinElute ccfDNA Kits	Varies†

* 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.

† See www.qiagen.com.

Accessories

15 ml/50 ml Tube Magnet	Magnet rack for 5 x 15 ml tubes and 3 x 50 ml tubes	36935
12-Tube Magnet	Magnet rack for 12 x 1.5/2 ml tubes	36912
AdnaMag-L	Magnetic rack for 8 tubes, 15 ml	399921
AdnaMag-S	Magnetic rack for 8 tubes, 1.5 ml	399911

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
08/2019	Modifications to protocol to improve reader comprehensibility.
01/2020	Updated text, ordering information and intended use for QIAcube Connect.
06/2020	Changed volume of Bead Elution Buffer, from 10 ml to 12 ml. Corrected typos in step 11 notes, pages 14 and 18: changed "step 9" to "step 10". "Quality Control" statement added.

Limited License Agreement for QIAamp MinElute ccfDNA Mini Kit and QIAamp MinElute ccfDNA Midi 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.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
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.

For updated license terms, see www.qiagen.com.

Trademarks: QIAGEN®, QIAcube®, Sample to Insight®, QIAamp®, MinElute® (QIAGEN Group); PAXgene® (PreAnalytiX GmbH); Bioanalyzer®, TapeStation® (Agilent Technologies, Inc.); Eppendorf®, Thermomixer® (Eppendorf AG); Streck® (Streck, Inc.); Vacutainer® (Becton Dickinson and Company). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

06/2020 HB-2366-004 © 2020 QIAGEN, all rights reserved.

Ordering www.qiagen.com/shop | Technical Support support.qiagen.com | Website www.qiagen.com