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QIAamp® UCP DNA Micro Handbook

For ultraclean DNA purification from small volume samples

Sample to Insight

QIAGEN

Contents

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QIAamp UCP DNA Micro Kit	**(50)**
Catalog no.	**56204**
Number of preps	**50**
QIAamp UCP MinElute® columns	50
Collection Tubes (2 ml)	150
Buffer AUT	50 ml
Buffer AUL	33 ml
Buffer AUW1	19 ml
Buffer AUW2	13 ml
Buffer AUE	12 ml
Proteinase K	1.25 ml
Quick-Start Protocol	1

Storage

QIAamp UCP MinElute columns should be stored at 2–8°C upon arrival. All buffers can be stored at room temperature (15–25°C) for up to 1 year without showing any reduction in performance. The proteinase K solution can be stored at room temperature or at 2–8°C.

Intended Use

The QIAamp UCP DNA Micro Kit is intended for ultraclean DNA purification from small volume samples. The QIAamp UCP DNA Micro Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

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

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

The sample-preparation waste contains guanidine hydrochloride from Buffers AUL and AUW1, 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 QIAamp UCP DNA Micro Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAamp UCP DNA Micro Kit provides fast and easy methods for purification of genomic, mitochondrial and microbial 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.

Special ultraclean treatment removes residual DNA contamination from the QIAamp UCP MinElute column. Stringent QC processes for columns and buffers ensure that there is no traceable contaminating microbial DNA at the time of kit delivery. This prevents DNA contamination from influencing downstream results, especially in the context of purification of DNA from very low biomass.

The procedure is suitable for a wide range of sample materials, such as small volumes of blood, plasma, serum, animal and human cells, swabs and small tissue samples. After sample lysis, the simple QIAamp UCP DNA Micro procedure, which is highly suited for simultaneous processing of multiple samples, yields pure, ultraclean DNA in less than 30 minutes. Flexible elution volumes down to 20 µl allow high concentration of nucleic acid. The eluted nucleic acids are ready for use in downstream applications including next-generation sequencing or storage at –30 to –15°C.

Principle and procedure

The QIAamp UCP DNA micro procedure consists of four steps (Figure 1):

- Lyse: The sample is lysed
- Bind: The DNA in the lysate binds to the membrane of the QIAamp UCP MinElute column
- Wash: The membrane is washed
- Elute: DNA is eluted from the membrane

Sample volumes

The QIAamp UCP DNA Micro Kit is designed for use with small amounts of sample. Protocols are provided for processing:

- Whole blood samples (1–100 µl)
- Animal or human cells (<1 x 10^6)
- Swabs
- Small amounts of tissue (<10 mg)
- Plasma and serum samples (up to 200 µl)

Sample lysis

Samples are lysed under highly denaturing conditions at elevated temperatures in the presence of proteinase K and Buffer AUT.

Binding DNA to the QIAamp UCP MinElute column membrane

To allow optimal binding of DNA to the membrane, Buffer AUL (and ethanol) is added to the lysate. Lysates are transferred onto a QIAamp UCP MinElute column, where DNA is adsorbed onto the silica-gel membrane as the lysate is drawn through by centrifugation. Salt and pH conditions ensure that proteins and other contaminants, which can inhibit enzymatic downstream reactions, are not retained on the QIAamp UCP MinElute column membrane. DNA yield depends on the volume or size and on the quality of the starting sample.

QIAamp UCP MinElute columns fit into most standard microcentrifuge tubes. Due to the volume of flow-through, 2 ml collection tubes are required to support the QIAamp UCP MinElute column during sample loading and wash steps.

Washing

While nucleic acids remain bound to the membrane of the QIAamp UCP MinElute column, contaminants are efficiently washed away during the wash steps.

DNA elution

DNA is eluted from the QIAamp UCP MinElute column using a small volume of Buffer AUE or Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10). Since the elution volume is small, the eluted DNA will be concentrated.

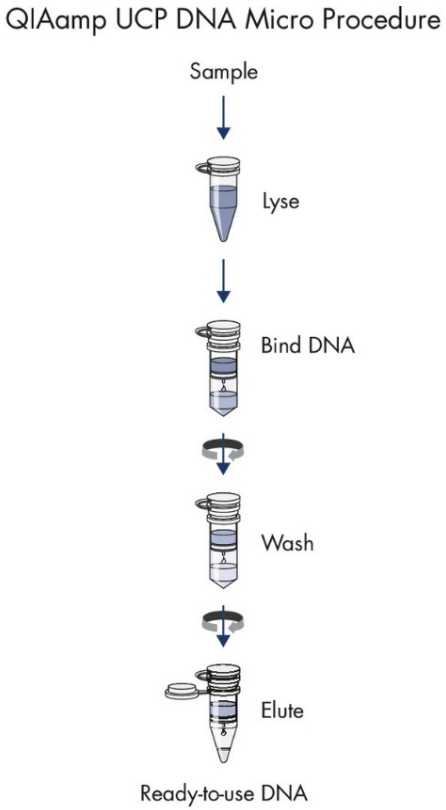

Figure 1. QIAamp UCP DNA Micro procedure.

Automated purification

Purification of DNA from low volume samples using the QIAamp UCP DNA Micro Kit can be fully automated on the QIAcube®. The innovative QIAcube uses advanced technology to process QIAGEN® spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), enabling you to continue using the QIAamp UCP DNA Micro Kit for purification of high-quality DNA. For more information about the automated procedure, see the relevant protocol sheet available at **www.qiagen.com/MyQIAcube**.

The QIAcube is 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/MyQIAcube**.

Figure 2. Automated DNA purification. DNA purification using the QIAamp UCP DNA Micro Kit can be automated on the QIAcube.

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 order to minimize contamination risks, use only highly pure, when possible DNA free materials and chemicals.

- Ethanol (96–100%)*
- **Optional**: If buffer AUE is incompatible with downstream applications, Microbial DNA-Free Water is recommended for DNA elution (cat. no. 338132).
- 1.5 ml and 2 ml microcentrifuge tubes
- Pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Disposable gloves
- Thermomixer, heated orbital incubator, heating block
- Microcentrifuge (with rotor for 2 ml tubes)
- Vortexer

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

Important Notes

DNA is present in every laboratory surrounding in the form of bacteria, viruses, human cells etc. During preparation, this DNA can be introduced into the sample. The QIAamp UCP DNA Micro Kit minimizes the risk of DNA contamination associated with the preparation of buffers and columns. However, DNA introduced during preparation from other sources can never be excluded. Use only highly pure, DNA-free materials and chemicals wherever possible. Avoid using materials that can potentially add sample-independent nucleic acids, such as carrier RNA. In addition, an appropriate control (mock preparation) must be included with every experiment in order to identify background DNA contaminations.

Elution of pure DNA

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

Buffer AUE or Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10) should be equilibrated to room temperature (15–25°C) before it is applied to the QIAamp UCP MinElute column. Yields will be increased with a second elution step. In order to increase the concentration of the DNA, the first eluate can be reapplied for the second elution.

Eluted DNA is collected in 1.5 ml elution tubes. If the purified 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. Low-bind tubes are beneficial for long-term storage.

Buffer AUE contains 10 mM Tris·Cl pH 8.3; 0.1 mM EDTA and 0.04% NaN_3 (sodium-azide). If any of those components or the pH value affects sensitive downstream applications, use Microbial DNA-Free Water for elution.

Yield and size of DNA

The yield of DNA isolated from biological samples strongly depends on the amount and source of the starting material. In addition, DNA size can also vary between 30 kb (in fresh samples) to 500 bp (in laser microdissections), or be below this range in forensic samples. If DNA yields are below 1 µg, quantification using a spectrophotometer will be difficult.

In this case, we recommend quantitative amplification methods (such as PCR) for determination of yield.

Handling of QIAamp UCP MinElute columns

Due to the sensitivity of many downstream applications, the following precautions are necessary when handling QIAamp UCP MinElute columns, to avoid cross-contamination between sample preparations:

- Carefully apply the sample or solution to the QIAamp UCP MinElute column. Pipet the sample into the QIAamp UCP MinElute column without wetting the rim of the column.
- Use aerosol-barrier pipet tips and always change pipet tips between liquid transfers.
- Avoid touching the QIAamp UCP 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 UCP 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 UCP MinElute columns will fit into most standard 1.5–2 ml microcentrifuge tubes. Additional 2 ml collection tubes are available separately.

All centrifugation steps should be carried out at room temperature (15–25°C).

Processing QIAamp UCP MinElute columns in a microcentrifuge

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

Preparation of RNA

When preparing viral RNA, work quickly during the manual steps of the procedure. If you have not previously worked with RNA, read “Appendix B: Handling RNA”, page 38 before starting.

Buffer AUE contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase inhibitors, it will not actively inhibit RNases introduced by inappropriate handling. Extreme care should be taken to avoid contamination with RNases when handling Buffer AUE.

Storage of serum and plasma samples

After collection and centrifugation, plasma or serum can be stored at 2–8°C for up to 6 hours. For long-term storage, freezing at –30 to –15°C or –90 to –60°C in aliquots is recommended.

Frozen plasma or serum samples must not be thawed more than once. Repeated freeze–thaw cycles lead to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. In addition, cryoprecipitates formed during freeze–thawing will clog the QIAamp UCP MinElute membrane. If cryoprecipitates are visible, they can be pelleted by centrifugation at 6800 x *g* for 3 minutes. The cleared supernatant should be removed and processed immediately without disturbing the pellet. This step will not reduce viral titers.

Preparation of buffers

Preparing Buffer AUT

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

Preparing Buffer AUL*

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

* Contains a chaotropic salt. Take appropriate laboratory safety measure and wear gloves when handling. Not compatible with disinfecting agents that contain bleach. See page 4 for safety information.

Preparing Buffer AUW1*

Add 25 ml ethanol (96–100%) to the bottle containing 19 ml Buffer AUW1 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AUW1 can be stored at room temperature (15–25°C) for up to 1 year.

Note: Before starting the procedure, mix the reconstituted Buffer AUW1 by shaking.

Preparing Buffer AUW2†

Add 30 ml ethanol (96–100%) to the bottle containing 13 ml Buffer AUW2 concentrate. Reconstituted Buffer AUW2 can be stored at room temperature (15–25°C) for up to 1 year.

Note: Before starting the procedure, mix the reconstituted Buffer AUW2 by shaking.

* Contains a chaotropic salt. Take appropriate laboratory safety measure and wear gloves when handling. Not compatible with disinfecting agents that contain bleach. See page 4 for safety information.

† Contains sodium azide.

Protocol: Isolation of Genomic DNA from Small Volumes of Blood

This protocol is for isolation of genomic DNA from 1–100 µl of whole blood treated with EDTA, citrate or heparin-based anticoagulants.

Important point before starting

- Perform all centrifugation steps at room temperature (15–25°C).

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Equilibrate Buffer AUE or Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10) for elution to room temperature.
- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- Ensure that Buffers AUW1 and AUW2 have been prepared according to the instructions on page 15.
- If Buffer AUL or Buffer AUT contains precipitates, dissolve by heating to 70°C with gentle agitation.

Procedure

1. Pipet 1–100 µl whole blood into a 1.5 ml microcentrifuge tube (not supplied). Add Buffer AUT to a final volume of 100 µl.
2. Add 10 µl proteinase K.
3. Add 100 µl Buffer AUL, close the lid and mix by pulse-vortexing for 15 s.

   To ensure efficient lysis, it is essential that the sample, Buffer AUT, proteinase K and Buffer AUL are thoroughly mixed to yield a homogeneous solution.

A white precipitate may form when Buffer AUL is added to Buffer AUT. The precipitate does not interfere with the QIAamp UCP DNA Micro procedure and will dissolve during the heat incubation in step 4.

4. Incubate at 56°C for 10 min.

   Note: If samples are shaken during the incubation, DNA yields can be increased.

5. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.
6. Add 50 µl ethanol (96–100%), close the lid and mix thoroughly by pulse-vortexing for 15 s. Incubate for 3 min at room temperature.

   Note: If room temperature exceeds 25°C, cool the ethanol on ice before adding to the tube.

7. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.
8. Carefully transfer the entire lysate from step 7 to the QIAamp UCP 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 UCP 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 until the QIAamp UCP MinElute column is empty.
9. Carefully open the QIAamp UCP MinElute column and add 500 µl Buffer AUW1 without wetting the rim. Close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
10. Carefully open the QIAamp UCP MinElute column and add 500 µl Buffer AUW2 without wetting the rim. Close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP MinElute column in a clean 2 ml collection tube and discard the collection tube containing the flow-through.

Note: Contact between the QIAamp UCP 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 UCP MinElute column. Take care when removing the QIAamp UCP MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp UCP MinElute column.

11. Centrifuge the QIAamp UCP MinElute column for 3 min at full speed (20,000 x *g*; 14,000 rpm) to dry the membrane completely. This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
12. Place the QIAamp UCP MinElute column in a clean 1.5 ml microcentrifuge tube (not supplied) and discard the collection tube containing the flow-through
13. Carefully open the lid of the QIAamp UCP MinElute column and apply 20–100 µl Buffer AUE or Microbial DNA-Free Water to the center of the membrane.

    If any of the components of Buffer AUE (see page 11 for details) or high pH interfere with downstream applications, use Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10) for elution.

    Important: Ensure that Buffer AUE or Microbial DNA-Free Water is equilibrated to room temperature (15–25°C). If using small elution volumes (<50 µl), dispense Buffer AUE or Microbial DNA-Free Water onto the center of the membrane to ensure complete elution of bound DNA. Remember that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.
14. Close the lid and incubate at room temperature (15–25°C) for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.

    Incubating the QIAamp UCP MinElute column loaded with Buffer AUE or Microbial DNA-Free Water for 5 min at room temperature before centrifugation generally increases DNA yield.
15. Repeat steps 13 and 14 to increase yield.

    Note: In order to increase the concentration, the first eluate can be re-applied for the second elution.

Protocol: Isolation of Genomic DNA from Cultured Mammalian Cells

This protocol is for isolation of genomic DNA from <1 x 10^6 cultured cells per preparation.

Important point before starting

- Perform all centrifugation steps at room temperature (15–25°C).

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Equilibrate Buffer AUE or Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10) for elution to room temperature.
- Set a thermomixer or heated orbital incubator to 56°C for use in step 4.
- Ensure that Buffers AUW1 and AUW2 have been prepared according to the instructions on page 15.
- If Buffer AUL or Buffer AUT contains precipitates, dissolve by heating to 70°C with gentle agitation.

Procedure

1. Pipet 1–100 µl media containing the cells into a 1.5 ml microcentrifuge tube (not supplied). Add Buffer AUT to a final volume of 100 µl.

   Note: If the sample volume exceeds 100 µl, centrifuge at 300 x *g* for 5 min and resuspend the pellet in 100 µl culture medium or PBS (not supplied).
2. Add 10 µl proteinase K.
3. Add 100 µl Buffer AUL, close the lid and mix by pulse-vortexing for 15 s.

   Note: To ensure efficient lysis, it is essential that the sample, Buffer AUT, proteinase K and Buffer AUL are thoroughly mixed to yield a homogeneous solution.

A white precipitate may form when Buffer AUL is added to Buffer AUT. The precipitate does not interfere with the QIAamp UCP DNA Micro procedure and will dissolve during the heat incubation in step 4.

4. Incubate at 56°C for 10 min.

   Note: If samples are shaken during the incubation, DNA yields can be increased.

5. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.
6. Add 50 µl ethanol (96–100%), close the lid and mix thoroughly by pulse-vortexing for 15 s. Incubate for 3 min at room temperature.

   Note: If room temperature exceeds 25°C, cool the ethanol on ice before adding to the tube.

7. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.
8. Carefully transfer the entire lysate from step 7 to the QIAamp UCP 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 UCP 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 until the QIAamp UCP MinElute column is empty.
9. Carefully open the QIAamp UCP MinElute column and add 500 µl Buffer AUW1 without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
10. Carefully open the QIAamp UCP MinElute column and add 500 µl Buffer AUW2 without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP MinElute column in a clean 2 ml collection tube and discard the collection tube containing the flow-through.

Note: Contact between the QIAamp UCP 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 UCP MinElute column. Take care when removing the QIAamp UCP MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp UCP MinElute column.

11. Centrifuge the QIAamp UCP MinElute column for 3 min at full speed (20,000 x *g*; 14,000 rpm) to dry the membrane completely. This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
12. Place the QIAamp UCP MinElute column in a clean 1.5 ml microcentrifuge tube (not supplied) and discard the collection tube containing the flow-through.
13. Carefully open the lid of the QIAamp UCP MinElute column and apply 20–100 µl Buffer AUE or Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10) to the center of the membrane.

    Note: If any of the components of Buffer AUE (see page 11 for details) or high pH interfere with downstream applications, use Microbial DNA-Free Water for elution.

    Important: Ensure that Buffer AUE or Microbial DNA-Free Water is equilibrated to room temperature (15–25°C). If using small elution volumes (<50 µl), dispense Buffer AUE or Microbial DNA-Free Water onto the center of the membrane to ensure complete elution of bound DNA. Remember that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.
14. Close the lid and incubate at room temperature (15–25°C) for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.

    Note: Incubating the QIAamp UCP MinElute column loaded with Buffer AUE or Microbial DNA-Free Water for 5 min at room temperature before centrifugation generally increases DNA yield.
15. Repeat steps 13 and 14 to increase yield.

    Note: In order to increase the concentration, the first eluate can be re-applied for the second elution.

Protocol: Isolation of Total DNA from Swabs

This protocol is for isolation of total (genomic and mitochondrial) DNA from buccal swabs, surface swabs, blood swabs and saliva swabs.

Important point before starting

- Perform all centrifugation steps at room temperature (15–25°C).

Things to do before starting

- Equilibrate Buffer AUE or Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10) for elution to room temperature (15–25°C).
- Set a thermomixer or heated orbital incubator to 56°C for use in step 3 and optionally in step 16 and a second thermomixer or heated orbital incubator to 70°C for use in step 6.
- If Buffer AUL or Buffer AUT contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffers AUW1 and AUW2 have been prepared according to the instructions on page 15.

Procedure

1. Place the swab in a 2 ml microcentrifuge tube (not supplied).

   If using an Omni Swab, eject the swab by pressing the end of the stem toward the swab. If using a cotton or Dacron swab, separate the swab from its shaft by hand or by using scissors.
2. Add 20 µl proteinase K and either 600 µl Buffer AUT (Omni Swabs) or 400 µl Buffer AUT (cotton or Dacron swabs), close the lid and mix by pulse-vortexing for 10 s.
3. Place the 2 ml tube in a thermomixer or heated orbital incubator, and incubate at 56°C with shaking at 900 rpm for at least 1 h.

   If using a heating block, vortex the tube for 10 s every 10 min to improve lysis.

4. Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.
5. Add either 600 µl Buffer AUL (Omni Swabs) or 400 µl Buffer AUL (cotton or Dacron swabs), close the lid and mix by pulse-vortexing for 15 s.

   Note: To ensure efficient lysis, it is essential that the sample and Buffer AUL are thoroughly mixed to yield a homogeneous solution. A white precipitate may form when Buffer AUL is added to Buffer AUT. The precipitate does not interfere with the QIAamp UCP DNA Micro procedure and will dissolve during incubation in step 6.
6. Place the 2 ml tube in a thermomixer or heated orbital incubator, and incubate at 70°C with shaking at 900 rpm for 10 min.

   If using a thermoblock, vortex the tube for 10 s every 3 min to improve lysis.
7. Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.
8. Add either 300 µl ethanol (96–100%) (Omni Swabs) or 200 µl ethanol (96–100%) (cotton or Dacron swabs), close the lid and mix by pulse-vortexing for 15 s.

   Note: To ensure efficient binding in step 10, it is essential that the sample and ethanol are thoroughly mixed to yield a homogeneous solution.
9. Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.
10. For Omni Swabs, follow step 10a. For cotton or Dacron swabs, follow step 10b.

    10a. Carefully transfer 700 µl lysate from step 9 to the QIAamp UCP 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. Carefully discard the flow-through from the collection tube and then place the QIAamp UCP MinElute column back into the collection tube. Carefully apply the remaining lysate from step 9 to the QIAamp UCP MinElute column without wetting the rim, close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP 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 UCP MinElute column is empty.

10b. Carefully transfer the entire lysate from step 9 to the QIAamp UCP 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 UCP 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 UCP MinElute column is empty.

11. Carefully open the QIAamp UCP MinElute column and add 500 µl Buffer AUW1 without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP MinElute column in a clean 2 ml collection tube and discard the collection tube containing the flow-through.

12. Carefully open the QIAamp UCP MinElute column and add 700 µl Buffer AUW2 without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Discard the flow-through and re-use the collection tube for step 13.

Note: Contact between the QIAamp UCP 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 UCP MinElute column. Take care when removing the QIAamp UCP MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp UCP MinElute column.

13. Carefully open the QIAamp UCP MinElute column and add 700 µl of ethanol (96–100%) without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP MinElute column in a clean 2 ml collection tube and discard the collection tube containing the flow-through.

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

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

15. Place the QIAamp UCP MinElute column in a clean 1.5 ml microcentrifuge tube (not supplied) and discard the collection tube containing the flow-through.

16. Carefully open the lid of the QIAamp UCP MinElute column and incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.

17. Apply 20–100 µl Buffer AUE or Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10) to the center of the membrane.

    Note: If any of the components of Buffer AUE (see page 11 for details) or the pH interfere with downstream applications, use Microbial DNA-Free Water for elution.

    Important: Ensure that Buffer AUE or Microbial DNA-Free Water is equilibrated to room temperature. Dispense Buffer AUE or Microbial DNA-Free Water onto the center of the membrane to ensure complete elution of bound DNA.

    QIAamp UCP MinElute columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application.

    Elution with small volumes increases the final DNA concentration in the eluate significantly, but reduces the overall DNA yield. Remember that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.

18. 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.

    Note: Incubating the QIAamp UCP MinElute column loaded with Buffer AUE or Microbial DNA-Free Water for 5 min at room temperature before centrifugation generally increases DNA yield.

19. Repeat steps 17 and 18 to increase yield.

    Note: In order to increase the concentration, the first eluate can be re-applied for the second elution.

Protocol: Isolation of Genomic DNA from Tissues

This protocol is for isolation of genomic DNA from less than 10 mg tissue.

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C)
- Prepare tissue samples on a cold surface (e.g., a glass, steel or aluminum plate placed on top of a block of dry ice).
- If using frozen tissue, ensure that the sample does not thaw out before addition of Buffer AUT in step 2.

Things to do before starting

- Equilibrate Buffer AUE or Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10) for elution to room temperature (15–25°C).
- Set a thermomixer or heated orbital incubator to 56°C for use in step 4, and a second thermomixer or heated orbital incubator to 70°C for use in step 5.
- If Buffer AUL or Buffer AUT contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffers AUW1 and AUW2 have been prepared according to the instructions on page 15.

Procedure

1. Transfer a tissue sample of less than 10 mg in weight to a 1.5 ml microcentrifuge tube (not supplied).

   Note: Be careful not to use too much tissue as lysates from 10 mg or more may clog the QIAamp UCP MinElute column.
2. Immediately add 180 µl Buffer AUT and equilibrate to room temperature (15–25°C).

3. Add 20 µl proteinase K and mix by pulse-vortexing for 15 s.
4. Place the 1.5 ml tube in a thermomixer or heated orbital incubator and incubate at 56°C overnight until the sample is completely lysed.

   Note: For small amounts of tissue, lysis is complete in 4–6 h, but best results are achieved after overnight lysis.
5. Add 200 µl Buffer AUL to the sample, mix by pulse-vortexing for 15 s, and incubate at 70°C for 10 min. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.

   Note: It is essential that the sample and Buffer AUL are mixed thoroughly to yield a homogeneous solution.

   A white precipitate may form on addition of Buffer AUL, which in most cases will dissolve during incubation at 70°C. The precipitate does not interfere with the procedure or with any subsequent application.
6. Add 200 µl ethanol (96–100%), close the lid and mix thoroughly by pulse-vortexing for 15 s. Incubate for 5 min at room temperature (15–25°C).

   Note: If room temperature exceeds 25°C, cool the ethanol on ice before adding to the tube.
7. Briefly centrifuge the 1.5 ml tube to remove drops from inside the lid.
8. Carefully transfer the entire lysate from step 7 to the QIAamp UCP 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 UCP 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 UCP MinElute column is empty.
9. Carefully open the QIAamp UCP MinElute column and add 500 µl Buffer AUW1 without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP MinElute column in a clean 2 ml collection tube and discard the collection tube containing the flow-through.

10. Carefully open the QIAamp UCP MinElute column and add 500 µl Buffer AUW2 without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP MinElute column in a clean 2 ml collection tube and discard the collection tube containing the flow-through.

    Note: Contact between the QIAamp UCP 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 UCP MinElute column. Take care when removing the QIAamp UCP MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp UCP MinElute column.

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

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

12. Place the QIAamp UCP MinElute column in a clean 1.5 ml microcentrifuge tube (not supplied) and discard the collection tube containing the flow-through.

13. Carefully open the lid of the QIAamp UCP MinElute column and apply 20–100 µl Buffer AUE or Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10) to the center of the membrane.

    Note: If any of the components of Buffer AUE (see page 11 for details) or the pH interfere with downstream applications, use Microbial DNA-Free Water for elution.

    Important: Ensure that Buffer AUE or Microbial DNA-Free Water is equilibrated to room temperature (15–25°C). If using small elution volumes (<50 µl), dispense Buffer AUE or Microbial DNA-Free Water onto the center of the membrane to ensure complete elution of bound DNA. QIAamp UCP MinElute columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. Remember that the volume of eluate will be up to 5 µl less than the volume of the solution applied to the column.

14. Close the lid and incubate at room temperature (15–25°C) for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.

    Note: Incubating the QIAamp UCP MinElute column loaded with Buffer AUE or Microbial DNA-Free Water for 5 min at room temperature before centrifugation generally increases DNA yield.

15. Repeat steps 13 and 14 to increase yield.

    Note: In order to increase the concentration, the first eluate can be re-applied for the second elution.

Protocol: Purification of Viral Nucleic Acids from Plasma or Serum

This protocol is for purification of viral nucleic acids from 200 µl of plasma or serum using the QIAamp UCP DNA Micro Kit and a microcentrifuge.

Important point before starting

- All centrifugation steps are carried out at room temperature (15–25°C).

Things to do before starting

- Equilibrate samples to room temperature.
- Equilibrate Buffer AUE or Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10) for elution to room temperature (15–25°C).
- Prepare a 56°C heating block for use in steps 4 and 13.
- Ensure that Buffers AUW1 and AUW2 have been prepared according to the instructions on page 15.

Procedure

1. Pipet 20 µl proteinase K into a 1.5 ml microcentrifuge tube (not provided).
2. Add 200 µl of plasma or serum into the microcentrifuge tube.

   If the sample volume is less than 200 µl, add the appropriate volume of Buffer AUT to bring the volume of the sample up to a total of 200 µl.
3. Add 200 µl Buffer AUL. Close the cap and mix by pulse-vortexing for 15 s.

   In order to ensure efficient lysis, it is essential that the sample and Buffer AUL are mixed thoroughly to yield a homogeneous solution.

   Note: Do not add proteinase K directly to Buffer AUL.
4. Incubate at 56°C for 15 min in a heating block.

5. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
6. Add 250 µl ethanol (96–100%) to the sample, close the cap and mix thoroughly by pulse-vortexing for 15 s. Incubate the lysate with the ethanol for 5 min at room temperature (15–25°C).

   Note: If ambient temperature exceeds 25°C, ethanol should be cooled on ice before adding to the lysate.
7. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
8. Carefully apply all of the lysate from step 7 onto the QIAamp UCP MinElute column without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP MinElute column in a clean 2 ml collection tube and discard the collection tube containing the filtrate. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp UCP MinElute column is empty.
9. **Recommended**: Carefully open the QIAamp UCP MinElute column, and add 500 µl Buffer AUW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp UCP MinElute column in a clean 2 ml collection tube and discard the collection tube containing the filtrate.

   Note: This step increases kit performance when processing inhibitory samples.
10. Carefully open the QIAamp UCP MinElute column and add 500 µl of Buffer AUW2 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Empty and re-use the collection tube containing the filtrate for step 11.
11. Carefully open the QIAamp UCP MinElute column and add 500 µl of ethanol (96–100%) without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the collection tube containing the filtrate. Ethanol carryover into the eluate may cause problems in downstream applications.

    Note: Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains ethanol, contacting the QIAamp UCP MinElute column. Removing the QIAamp UCP MinElute column and collection tube from the rotor may also cause flow-through to come into contact with the QIAamp UCP MinElute column.

12. Place the QIAamp UCP MinElute column in a clean 2 ml collection tube. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min to dry the membrane completely.
13. **Recommended**: Place the QIAamp UCP MinElute column into a new 2 ml collection tube (not provided), open the lid and incubate the assembly at 56°C for 3 min to dry the membrane completely. This step serves to evaporate any remaining liquid.
14. Place the QIAamp UCP MinElute column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube with the filtrate. Carefully open the lid of the QIAamp UCP MinElute column, and apply 20–150 µl Buffer AUE or Microbial DNA-Free Water (see "Equipment and Reagents to Be Supplied by User", page 10) to the center of the membrane. 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.

    Important: Ensure that the elution buffer is equilibrated to room temperature. If elution is done in small volumes (<50 µl), the elution buffer must be dispensed onto the center of the membrane for complete elution of bound RNA and DNA.

    Elution volume is flexible and can be adapted according to the requirements of the downstream application. Remember that the recovered eluate volume will be approximately 5 µl less than the elution buffer volume applied onto the column. Incubating the QIAamp UCP MinElute column loaded with Buffer AUE or water for 5 min at room temperature before centrifugation generally increases DNA and RNA 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/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
Little or no DNA in the eluate		
a)	Samples were frozen and thawed more than once	Avoid repeated freezing and thawing of samples. Where possible, always use fresh samples or samples that have been thawed only once.
b)	Samples were kept for too long at room temperature	DNA in the samples may degrade during prolonged storage at room temperature. Where possible, always use fresh samples, or store the samples at 2–8°C (non-dried blood) or at –20°C (tissue samples). Dried blood spots or stains can be stored at room temperature in the dark without significant DNA degradation.
c)	Insufficient mixing with Buffer AUL	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer AUL immediately and thoroughly by pulse-vortexing.
d)	Insufficient sample lysis in Buffer AUL	Proteinase K was stored at high temperatures for a prolonged time. Repeat the procedure using new samples and fresh proteinase K.
e)	No alcohol added to the lysate before loading onto the QIAamp UCP MinElute column	Repeat the purification procedure with a new sample.
f)	Low-percentage ethanol was used	Repeat the purification procedure with new samples and 96–100% ethanol.
g)	Buffer AUW1 or AUW2 was prepared incorrectly	Check that the Buffer AUW1 and Buffer AUW2 concentrates were diluted with the correct volume of 96–100% ethanol. Repeat the purification procedure with new samples, if available.
h)	DNA was not efficiently eluted	To increase elution efficiency, pipet Buffer AUE onto the QIAamp UCP MinElute column and incubate the column for 5 minutes at room temperature before centrifugation.

		Comments and suggestions
A_{260}/A_{280} ratio for purified nucleic acids is low		
a)	Insufficient mixing with Buffer AUL	Repeat the procedure with a new sample. Be sure to mix the sample and Buffer AUL immediately and thoroughly by pulse-vortexing.
b)	Decreased proteinase K activity	Repeat the DNA purification procedure with a new sample and with new proteinase K.
c)	No alcohol added to the lysate before loading onto the QIAamp UCP MinElute column	Repeat the purification procedure with a new sample.
d)	Buffer AUW1 or Buffer AUW2 prepared with low-percentage ethanol	Check that Buffer AUW1 and Buffer AUW2 concentrates were diluted with 96–100% ethanol. Repeat the purification procedure with a new sample.
e)	Buffer AUW1 or Buffer AUW2 prepared incorrectly	Check that Buffer AUW1 and Buffer AUW2 concentrates were diluted with correct volumes of pure ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification procedure with a new sample.
f)	Buffer AUW1 and Buffer AUW2 used in the wrong order	Ensure that Buffer AUW1 and Buffer AUW2 are used in the correct order in the protocol. Add 1 volume of Buffer AUL and 1 volume of ethanol to the eluate, and continue with step 8 of "Protocol: Isolation of Genomic DNA from Small Volumes of Blood", page 16.
DNA or RNA does not perform well in downstream applications		
a)	Little or no DNA in the eluate	See "Little or no DNA in the eluate", above.
b)	Reduced sensitivity of amplification reaction	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 adjusted proportionally. Use Microbial DNA-Free Water instead of Buffer AUE for elution.
c)	Performance of purified nucleic acids in assays varies with aging of reconstituted wash buffers	Salt and ethanol components of wash Buffers AUW1 and AUW2 may have separated out after being unused for a long period. Always mix buffers thoroughly before each purification procedure.
d)	Inhibitory substances in preparation	See "A_{260}/A_{280} ratio for purified nucleic acids is low" (above) for possible reasons. Increase the eluate volume to 100 µl, if necessary, and repeat the purification procedure from step 8 of "Protocol: Isolation of Genomic DNA from Small Volumes of Blood", page 16).

		Comments and suggestions
General handling		
a)	Clogged QIAamp UCP MinElute column	Incomplete lysis caused clogging of the membrane. Increase the lysis time to fully lyse the sample. Cryoprecipitates may have formed in plasma due to repeated freezing and thawing. These can block the QIAamp UCP MinElute column. Do not use plasma that has been frozen and thawed more than once. If cryoprecipitates have formed, clear the sample by centrifugation as described in "Storage of serum and plasma samples" on page 14 before starting the sample preparation.
b)	Lysate not completely passed through silica membrane	Centrifuge for 1 minute at full speed or until all the lysate has passed through the membrane.
c)	Eluted DNA is contaminated with background DNA	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. Use highly pure materials and reagents (DNA-free, if possible). Run appropriate (water) controls.

Appendix A: Determination of Concentration, Yield, Purity and Length of DNA

Determination of concentration, yield, and purity

DNA yields are determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9. Absorbance readings at 260 nm should lie between 0.1 and 1.0 to be accurate.

Sample dilution should be adjusted accordingly. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220–320 nm (a scan will show if there are other factors affecting absorbance at 260 nm). Both DNA and RNA are measured with a spectrophotometer. To measure only DNA, a fluorometer must be used.

If DNA yields are below 1 µg, quantification using a spectrophotometer will be difficult. In this case, we recommend quantitative amplification methods for determination of yield.

Determination of DNA length

The length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. The DNA should be concentrated by alcohol* precipitation and reconstituted by gentle agitation in approximately 30 µl TE buffer, pH 8.0,* for at least 30 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room temperature since over-dried genomic DNA is very difficult to redissolve. Load 3–5 µg DNA per well.

Standard PFGE conditions are as follows:

- 1% agarose gel in 0.5x TBE electrophoresis buffer*
- Switch intervals: 5–40 s
- Run time: 17 h
- Voltage: 170 V

* 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.

Appendix B: Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and only minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non-disposable vessels and solutions while working with RNA.

General handling

Proper microbiological aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase 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 procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by RNase-free water* (see "Solutions", page 40). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with detergent, thoroughly rinsed and oven-baked at >240°C for four or more hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Oven baking will both inactivate ribonucleases and ensure that no other nucleic acids (such as plasmid DNA) remain on the surface of the glassware. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Cover the glassware with 0.1% DEPC in water overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to remove residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS)*, rinsed with water, dried with ethanol*† and then filled with a solution of 3% hydrogen peroxide*. After 10 minutes at room temperature, the electrophoresis tanks should be rinsed thoroughly with RNase-free water.

* 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.

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first and then dissolve Tris to make the appropriate buffer.

DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be removed from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution or let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. It may be desirable to test water sources for the presence of contaminating RNases since many sources of distilled water are free of RNase activity.

Ordering Information

Product	Contents	Cat. no.
QIAamp UCP DNA Micro Kit (50)	For 50 preps: QIAamp UCP MinElute spin columns, QIAGEN Proteinase K, Buffers	56204
QIAcube and QIAcube accessories – for fully automated sample preparation using QIAGEN spin-column kits		
QIAcube (110 V)*† (230 V)‡	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits: includes installation and training, 1-year warranty on parts and labor	9001292*† 9001293‡
Starter Pack, QIAcube	Pack includes: reagent bottle racks (3); rack labeling strips (8); 200 µl filter-tips (1024); 1000 µl filter-tips (2048); 30 ml reagent bottles (18); rotor adapters (240); rotor adapter holder; 1.5 ml elution tubes (240)	990395
Related products		
Microbial DNA-Free Water	12 tubes of 1.35 ml each	338132
RNeasy® UCP Micro Kit (50)	50 RNeasy UCP MinElute Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free DNase I, RNase-free Reagents and UCP Buffers	73934
Collection Tubes (2 ml)	1000 collection tubes (2 ml)	19201

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.

* US and Canada; † Japan; ‡ Rest of world

Revision History

Table 1 details the revision history of the *QIAamp UCP DNA Micro Handbook*.

Table 1. Revision history

Document	Changes
HB-2174-001	Initial release
HB-2174-002	New section: "Storage of serum and plasma samples" on page 14. New protocol: "Protocol: Purification of Viral Nucleic Acids from Plasma or Serum" on page 30. New Appendix: "Appendix B: Handling RNA" on page 38. Throughout: Substitution of "ultraclean PCR water" for "Microbial DNA-Free Water".

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