August 2003

QIAamp® DNA Micro Handbook

For purification of genomic DNA from

small volumes of blood
dried blood spots
swabs
forensic case work samples
chewing gum
urine
tissues
laser-microdissected tissues

For cleanup of genomic DNA

QIAGEN

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QIAamp Kits are intended as general-purpose devices. No claim or representation is intended for their use to identify any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of QIAamp Kits for any particular use, since the performance characteristics of these kits have not been validated for any specific organism. QIAamp Kits may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Contents

Kit Contents

QIAamp® DNA Micro Kit Catalog no. Number of preps	(50) 56304 50
QIAamp MinElute™ Columns	50
Collection Tubes (2 ml)	200
Buffer ATL	50 ml
Buffer AL*	33 ml
Buffer AW1* (concentrate)	19 ml
Buffer AW2† (concentrate)	13 ml
Buffer AE	12 ml
Carrier RNA (red cap)	310 µg
Proteinase K	1.25 ml
Handbook	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for safety information.

† Contains sodium azide as a preservative.

Storage

QIAamp MinElute Columns should be stored at 2–8°C upon arrival. However, short-term storage (up to 4 weeks) at room temperature (15–25°C) does not affect their performance.

All buffers can be stored at room temperature (15–25°C).

QIAamp DNA Micro Kits contain a novel, ready-to-use Proteinase K solution, which is supplied in a specially formulated storage buffer. Proteinase K is stable for at least one year after delivery when stored at room temperature (15–25°C). For storage longer than one year or if ambient temperatures often exceed 25°C, we suggest storing Proteinase K at 2–8°C.

Quality Control

As part of the stringent QIAGEN® quality assurance program, the performance of QIAamp DNA Micro Kits is monitored routinely on a lot-to-lot basis. All components are tested separately to ensure highest performance and reliability.

Product Use Limitations

QIAamp Kits are intended as general-purpose devices. No claim or representation is intended for their use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of QIAamp Kits for any particular use, since the performance characteristics of these kits have not been validated for any specific organism. QIAamp Kits may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

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.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back page).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QIAamp DNA Micro Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back page).

Safety Information

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

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

Buffers AL and AW1 contain guanidine hydrochloride, 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.

The following risk and safety phrases apply to components of the QIAamp DNA Micro Kit:

Buffer AL and Buffer AW1

Contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* R22-36/38, S13-26-36-46

Proteinase K

Contains proteinase K: sensitizer, irritant. Risk and safety phrases:* R36/37/38-42/43, S23-24-26-36/37

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R22: Harmful if swallowed; R36/38: Irritating to eyes and skin; R36/37/38: Irritating to eyes, respiratory system, and skin; R42/43: May cause sensitization by inhalation and skin contact; S13: Keep away from food, drink, and animal feeding stuffs; S23: Do not breathe spray; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37: Wear suitable protective clothing and gloves; S46: If swallowed, seek medical advice immediately and show this container or label.

Introduction

The QIAamp DNA Micro Kit uses well-established technology for purification of genomic and mitochondrial DNA from small sample volumes or sizes. The kit combines the selective binding properties of a silica-based membrane with flexible elution volumes of between 20 and 100 µl. The procedure is suitable for a wide range of sample materials such as small volumes of blood, blood cards, small tissue samples, and forensic samples.

The procedure is designed to ensure that there is no sample-to-sample cross-contamination and allows safe handling of potentially infectious samples. After sample lysis, the simple QIAamp DNA Micro procedure, which is highly suited for simultaneous processing of multiple samples, yields pure DNA in less than 30 minutes.

DNA is eluted in Buffer AE or water and is immediately ready for use in amplification reactions or for storage at –20°C. Purified DNA is free of proteins, nucleases, and other impurities.

Principle and procedure

The QIAamp DNA Micro procedure consists of 4 steps (see flowchart, page 9):

- Lyse — the sample is lysed
- Bind — the DNA in the lysate binds to the membrane of the QIAamp MinElute Column
- Wash — the membrane is washed
- Elute — DNA is eluted from the membrane

Sample volumes

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

- Whole blood samples (1–100 µl)
- Blood card punches (3 mm diameter)
- Single body fluid swabs
- Forensic samples, including 0.5 cm^2 body fluid stains, nail clippings, hair, and saliva on stamps, envelopes, and cigarette butts
- Chewing gum (up to 30 mg)
- Urine (up to 10 ml)
- Small amounts of tissue (<10 mg)
- Laser-microdissected tissues

In addition, the QIAamp DNA Micro Kit can be used to clean up genomic DNA samples of up to 100 µl containing up to 10 µg DNA.

Lysing samples

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

Binding DNA to the QIAamp MinElute Column membrane

To allow optimal binding of DNA to the membrane, Buffer AL (and in most protocols, ethanol) is added to the lysate. Lysates are transferred onto a QIAamp 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 PCR and other downstream enzymatic reactions, are not retained on the QIAamp MinElute Column membrane. DNA yield depends on the volume or size and on the quality of the starting sample.

For cleanup of genomic DNA, the conditions for binding to the silica-gel membrane are adjusted by adding Buffers AW1 and AW2.

QIAamp MinElute Columns fit into most standard microcentrifuge tubes. Due to the volume of flow-through, 2 ml collection tubes (provided) are required to support the QIAamp MinElute Column during sample loading and wash steps.

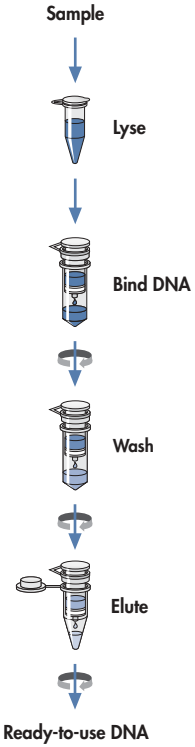
Washing away residual contaminants

While nucleic acids remain bound to the membrane of the QIAamp MinElute Column, contaminants are efficiently washed away using first Buffer AW1 and then Buffer AW2. For cleanup of genomic DNA, a single wash step with Buffer AW2 is sufficient to remove contaminants.

Eluting pure DNA

DNA is eluted from the QIAamp MinElute Column using a small volume of either Buffer AE or distilled water. Since the elution volume is small, the eluted DNA will be concentrated.

QIAamp DNA Micro Procedure



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 material safety data sheets (MSDSs), available from the product supplier.

- Ethanol (96–100%)
- 1.5 ml or 2 ml microcentrifuge tubes (for lysis steps)
- 1.5 ml microcentrifuge tubes (for elution steps) (available from Brinkmann [Safe-Lock, cat. no. 022363204], Eppendorf [Safe-Lock, cat. no. 0030 120.086], or Sarstedt [Safety Cap, cat. no. 72.690])*
- Pipet tips (to avoid cross contamination, we recommend pipet tips with aerosol barriers)
- Disposable gloves
- Thermomixer, heated orbital incubator, heating block, or water bath
- Microcentrifuge with rotor for 2 ml tubes
- Scissors
- Blood collection cards (if processing dried blood spots) (available from Schleicher & Schuell [903 paper or IsoCode® paper] and Whatman [Bloodstain Card or FTA® Card])*
- Paper punch for blood collection cards (available from Schleicher & Schuell)*
- Sterile Omni Swabs (available from Whatman), cotton swabs, or Dacron® swabs (if processing swab samples)
- QIAshredder (for maximum yields when processing swabs or stained fabrics)
- Dithiothreitol (DTT) (if processing hair, nail clippings, or semen)

* This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

Eluting pure DNA

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

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

The volume of eluate recovered may be up to 5 µl less than the volume of elution solution (i.e., Buffer AE or distilled water) applied to the QIAamp MinElute Column. For example, an elution volume of 20 µl results in ≥15 µl eluate. The volume of eluate recovered depends on the nature of the sample.

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

Eluted DNA can be collected in standard 1.5 ml microcentrifuge tubes (not provided). If the purified DNA is to be stored for up to 24 hours, we recommend storage at 2–8°C. For storage longer than 24 hours, we recommend elution in Buffer AE (10 mM Tris·Cl, 0.5 mM EDTA, pH 9.0) and storage at –20°C.

If high pH or EDTA affects sensitive downstream applications, use water for elution. However, ensure that the pH of the water is at least 7.0 (deionized water from certain sources can be acidic). DNA stored in water is subject to degradation by acid hydrolysis.

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) and 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 for determination of yield.

If purifying DNA from very small amounts of sample, such as 1–10 µl blood or forensic samples, we recommend adding carrier RNA to the sample. Be aware that the sample will then contain considerably more carrier RNA than DNA. Therefore, when quantifying the purified DNA, avoid quantification methods that are not specific for DNA.

Carrier RNA

The kit is supplied with carrier RNA, which can be added to Buffer AL if required. Carrier RNA enhances binding of DNA to the QIAamp MinElute Column membrane, especially if there are very few target molecules in the sample.

The amount of lyophilized carrier RNA provided is sufficient for the volume of Buffer AL supplied with the kit. The concentration of carrier RNA used in the QIAamp DNA Micro procedure allows the procedure to be used as a generic purification system compatible with many different amplification systems.

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. If carrier RNA is used, eluates from QIAamp MinElute Columns contain both sample DNA and carrier RNA, with the amount of carrier RNA greatly exceeding the amount of DNA. Calculations of how much eluate to add to downstream amplifications should therefore be based on the amount of carrier RNA added to Buffer AL. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA added to Buffer AL.

Handling of QIAamp MinElute Columns

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

- Carefully apply the sample or solution to the QIAamp MinElute Column. Pipet the sample into the QIAamp MinElute Column without wetting the rim of the column.
- Always change pipet tips between liquid transfers. We recommend the use of aerosol-barrier pipet tips.
- Avoid touching the QIAamp MinElute Column membrane with the pipet tip.
- After all pulse-vortexing steps, briefly centrifuge the microcentrifuge tubes to remove drops from the inside of the lids.
- Open only one QIAamp MinElute Column at a time, and take care to avoid generating aerosols.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

Centrifugation

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

Centrifugation of QIAamp MinElute Columns is performed at 6000 x *g* (8000 rpm) to reduce centrifuge noise. Centrifugation at full speed will not improve DNA yields.

However, centrifugation of QIAamp MinElute Columns at full speed is required at 2 steps in the procedure: the dry centrifugation step after the membranes are washed, and the elution step.

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

Processing QIAamp MinElute Columns in a microcentrifuge

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

Preparation of buffers

Preparing Buffer ATL

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

Preparing Buffer AL

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

Preparing Buffer AW1

Add 25 ml ethanol (96–100%) to the bottle containing 19 ml Buffer AW1 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 can be stored at room temperature (15–25°C) for up to 1 year.

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

Preparing Buffer AW2

Add 30 ml ethanol (96–100%) to the bottle containing 13 ml Buffer AW2 concentrate. Reconstituted Buffer AW2 can be stored at room temperature (15–25°C) for up to 1 year.

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

Adding carrier RNA to Buffer AL

For purification of DNA from very small amounts of sample, such as low volumes of blood (<10 µl) or forensic samples, we recommend adding carrier RNA to Buffer AL. For samples containing larger amounts of DNA, addition of carrier RNA is optional.

Add 310 µl Buffer AE to the tube containing 310 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

Calculate the volume of Buffer AL and dissolved carrier RNA needed per batch of samples by multiplying the number of samples to be **simultaneously** processed by the volumes given in Table 1. To allow for pipetting errors, always prepare enough buffer for processing two extra samples.

Gently mix Buffer AL and dissolved carrier RNA by inverting the tube 10 times. To avoid foaming, do not vortex. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL. Buffer AL containing carrier RNA is stable at room temperature (15–25°C) for up to 48 hours.

Table 1. Volumes of Buffer AL and dissolved carrier RNA required for one DNA preparation using the QIAamp DNA Micro Kit

Protocol for isolation of genomic DNA from	Volume of Buffer AL added to sample	Dissolved carrier RNA
Small volumes of blood (page 15)	100 µl	1 µl
Dried blood spots (page 18)	200 µl	1 µl
Swabs (page 21)	600 µl* or 400 µl†	1 µl
Chewing gum (page 25)	300 µl	1 µl
Forensic samples (page 28)	300 µl	1 µl
Urine (page 32)	300 µl	1 µl
Tissues (page 35)	200 µl	1 µl
Laser-microdissected tissues (page 38)	50 µl	1 µl

* If using ejectable swabs.

† If using non-ejectable swabs.

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 AE or distilled water for elution to room temperature.
- Set a thermomixer or heated orbital incubator to 56°C for use in step 5.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 13.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Optional: If processing low volume samples (<10 µl), add carrier RNA dissolved in Buffer AE to Buffer AL according to the instructions on page 14.

Procedure

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

   To ensure efficient lysis, it is essential that the sample, Buffer ATL, Proteinase K, and Buffer AL are thoroughly mixed to yield a homogeneous solution.

   Note: If the volume of blood is lower than 10 µl, we recommend adding carrier RNA to Buffer AL (see page 14). Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL.

   A white precipitate may form when Buffer AL is added to Buffer ATL. The precipitate does not interfere with the QIAamp procedure and will dissolve during the heat incubation in step 5.
5. **Incubate at 56°C for 10 min.**

   Note: If samples are shaken during the incubation, DNA yields can be increased.
6. **Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.**

7. **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.

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

9. **Carefully transfer the entire lysate from step 8 to the QIAamp MinElute Column without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

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

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

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

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

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

13. **Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute Column and apply 20–100 µl Buffer AE or distilled water to the center of the membrane.**

    If high pH or EDTA affects sensitive downstream applications, use water for elution (see page 11).

    Important: Ensure that Buffer AE or distilled water is equilibrated to room temperature (15–25°C). If using small elution volumes (<50 µl), dispense Buffer AE or distilled water onto the center of the membrane to ensure complete elution of bound DNA.

QIAamp MinElute Columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. 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 MinElute Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

Protocol: Isolation of Genomic DNA from Dried Blood Spots

This protocol is for isolation of genomic DNA from blood card punches with diameters of approx. 3 mm (1/8 inch). This protocol is suitable for untreated blood or blood treated with anticoagulants such as EDTA, citrate, or heparin. The blood must be spotted and dried on filter paper such as 903 paper or IsoCode paper (Schleicher & Schuell), Bloodstain Card or FTA Card (Whatman), Guthrie test cards, or comparable blood cards.

Important point before starting

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

Things to do before starting

- Equilibrate Buffer AE or distilled water for elution to room temperature (15–25°C).
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- 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 7. If thermomixers or heated orbital incubators are not available, heating blocks or water baths can be used instead.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 13.
- Optional: If processing very small amounts of starting material, add carrier RNA dissolved in Buffer AE to Buffer AL according to the instructions on page 14.

Procedure

1. **Cut 3 mm (1/8 inch) diameter punches from a dried blood spot with a single-hole paper punch. Place up to 3 blood card punches into a 1.5 ml microcentrifuge tube (not provided).**
2. **Add 180 µl Buffer ATL.**
3. **Add 20 µl Proteinase K and mix thoroughly by vortexing.**
4. **Place the 1.5 ml tube in a thermomixer or heated orbital incubator, and incubate at 56°C with shaking at 900 rpm for 1 h.**

   If using a heating block or water bath, vortex the tube for 10 s every 10 min to improve lysis.
5. **Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.**

6. **Add 200 µl Buffer AL, close the lid, and mix by pulse-vortexing for 10 s.**

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

   A white precipitate may form when Buffer AL is added to Buffer ATL. The precipitate does not interfere with the QIAamp procedure and will dissolve during the heat incubation in step 7.

   Note: If processing only 1 blood card punch with a diameter of 3 mm or less, we recommend adding carrier RNA to Buffer AL (see page 14). Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL.

7. **Place the 1.5 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 heating block or water bath, vortex the tube for 10 s every 3 min.

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

9. **Carefully transfer the entire lysate from step 8 to the QIAamp MinElute Column without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

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

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

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

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

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

13. **Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute Column and apply 20–100 µl Buffer AE or distilled water to the center of the membrane.**

    If high pH or EDTA affects sensitive downstream applications, use water for elution (see page 11).

    Important: Ensure that Buffer AE or distilled water is equilibrated to room temperature (15–25°C). If using small elution volumes (<50 µl), dispense Buffer AE or distilled water onto the center of the membrane to ensure complete elution of bound DNA.

    QIAamp MinElute Columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. 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 MinElute Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

Protocol: Isolation of Genomic DNA from Swabs

This protocol is for isolation of genomic DNA from sperm swabs, blood swabs, and saliva swabs.

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Check whether carrier RNA is required (see pages 12 and 14).

Things to do before starting

- Equilibrate Buffer AE or distilled water for elution to room temperature (15–25°C).
- Set a thermomixer or heated orbital incubator to 56°C for use in step 3, and a second thermomixer or heated orbital incubator to 70°C for use in step 6. If thermomixers or heated orbital incubators are not available, heating blocks or water baths can be used instead.
- If processing semen swabs, prepare an aqueous 1 M DTT (dithiothreitol) stock solution. Store aliquots at –20°C. Thaw immediately before use.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 13.
- Optional: To harvest lysate remaining in the swab, QIAshredder Spin Columns may be required.

Procedure

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

   If using an Omni Swab, eject the swab by pressing the end of the stem towards 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 ATL (if using an Omni Swab) or 400 µl Buffer ATL (if using a cotton or Dacron swab), 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 or water bath, 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 AL (if using an Omni Swab) or 400 µl Buffer AL (if using a cotton or Dacron swab), close the lid, and mix by pulse-vortexing for 15 s.**

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

   A white precipitate may form when Buffer AL is added to Buffer ATL. The precipitate does not interfere with the QIAamp procedure and will dissolve during incubation in step 6.

   Note: If carrier RNA is required (see page 12), add 1 µg dissolved carrier RNA to either 600 µl Buffer AL (if using an Omni Swab) or 400 µl Buffer AL (if using a cotton or Dacron swab). Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL.

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 or water bath, 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%) (if using an Omni Swab) or 200 µl ethanol (96–100%) (if using a cotton or Dacron swab), close the lid, and mix by pulse-vortexing for 15 s.**

   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. **If using an Omni Swab, follow step 10a. If using a cotton or Dacron swab, follow step 10b.**

10a. **Carefully transfer 700 µl lysate from step 9 to the QIAamp MinElute Column 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 MinElute Column back into the collection tube or transfer the QIAamp MinElute Column to a clean 2 ml collection tube. Carefully apply the remaining lysate from step 9 to the QIAamp MinElute Column without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

   Note: Up to 250 µl lysate remains in the swab. To harvest this remaining lysate, place the swab in a QIAshredder (not supplied) and centrifuge at full speed

(20,000 x *g*; 14,000 rpm) for 2 min. Transfer the flow-through to the QIAamp MinElute Column without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min.

10b. Carefully transfer the entire lysate from step 9 to the QIAamp MinElute Column without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

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

Note: Up to 200 µl lysate remains in the swab. To harvest this remaining lysate, place the swab in a QIAshredder (not supplied) and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 2 min. Transfer the flow-through to the QIAamp MinElute Column without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min.

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

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

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

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

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

14. Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute Column and apply 20–50 µl Buffer AE or distilled water to the center of the membrane.

If high pH or EDTA affects sensitive downstream applications, use water for elution (see page 11).

Important: Ensure that Buffer AE or distilled water is equilibrated to room temperature (15–25°C). Dispense Buffer AE or distilled water onto the center of the membrane to ensure complete elution of bound DNA.

QIAamp MinElute Columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. Remember that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.

15. **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 MinElute Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

Protocol: Isolation of Genomic DNA from Chewing Gum

This protocol is for isolation of genomic DNA from chewing gum.

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Check whether carrier RNA is required (see pages 12 and 14).

Things to do before starting

- Equilibrate Buffer AE or water for elution to room temperature (15–25°C).
- Set a thermomixer or heated orbital incubator to 56°C for use in step 3, and a second thermomixer or heated orbital incubator to 70°C for use in step 6. If thermomixers or heated orbital incubators are not available, heating blocks or water baths can be used instead.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 13.

Procedure

1. **Cut up to 30 mg of chewing gum into small pieces and transfer them to a 1.5 ml microcentrifuge tube (not provided).**
2. **Add 300 µl Buffer ATL and 20 µl Proteinase K, and mix by pulse-vortexing for 10 s.**
3. **Place the 1.5 ml tube in a thermomixer or heated orbital incubator, and incubate at 56°C with shaking at 900 rpm for at least 3 h.**

   If using a heating block or water bath, vortex the tube for 10 s every 30 min to improve lysis.
4. **Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.**
5. **Add 300 µl Buffer AL, close the lid, and mix by pulse-vortexing for 10 s.**

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

   A white precipitate may form when Buffer AL is added to Buffer ATL. The precipitate does not interfere with the QIAamp procedure and will dissolve during incubation in step 6.

   Note: If carrier RNA is required (see page 12), add 1 µg dissolved carrier RNA to 300 µl Buffer AL. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL.

6. **Place the 1.5 ml tube in the thermomixer or heated orbital incubator, and incubate at 70°C with shaking at 900 rpm for 1 h.**

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

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

8. **Add 150 µl ethanol (96–100%), close the lid, and mix by pulse-vortexing for 10 s.**

   To ensure efficient binding in step 10, it is essential that the sample and ethanol are thoroughly mixed to yield a homogeneous solution.

9. **Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.**

10. **Carefully transfer the supernatant from step 9 to the QIAamp MinElute Column without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

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

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

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

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

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

14. **Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute Column and apply 20–50 µl Buffer AE or distilled water to the center of the membrane.**

    If high pH or EDTA affects sensitive downstream applications, use water for elution (see page 11).

    Important: Ensure that Buffer AE or distilled water is equilibrated to room temperature (15–25°C). Dispense Buffer AE or distilled water onto the center of the membrane to ensure complete elution of bound DNA.

    QIAamp MinElute Columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. Remember that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.

15. **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 MinElute Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

Protocol: Isolation of Genomic DNA from Forensic Case Work Samples

This protocol is for isolation of genomic DNA from cigarette butts, envelopes, hair, nail clippings, stamps, or blood, saliva, or semen stains.

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Check whether carrier RNA is required (see pages 12 and 14).

Things to do before starting

- Equilibrate Buffer AE or distilled water for elution to room temperature (15–25°C).
- Set a thermomixer or heated orbital incubator to 56°C for use in step 2, and a second thermomixer or heated orbital incubator to 70°C for use in step 5. If thermomixers or heated orbital incubators are not available, heating blocks or water baths can be used instead.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- If processing semen stains, hair, or nail clippings, prepare an aqueous 1 M DTT (dithiothreitol) stock solution. Store aliquots at –20°C. Thaw immediately before use.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 13.
- Optional: If processing stained fabrics, QIAshredder Spin Columns may be required.

Procedure

1. Lyse samples according to step 1a (cigarette butts), 1b (envelopes or stamps), 1c (hair roots), 1d (hair shafts without roots), 1e (nail clippings), or 1f (material stained with blood, saliva, or semen).

1a. Lysing cigarette butts:

Cut out a 1 cm² piece of outer paper from the end of the cigarette or filter. Cut this piece into 6 smaller pieces. Transfer the pieces to a 1.5 ml microcentrifuge tube (not provided).

Add 300 µl Buffer ATL and 20 µl Proteinase K, close the lid, and mix by pulse-vortexing for 10 s. Continue the procedure from step 2.

1b. Lysing envelopes or stamps:

Cut out a 0.5–2.5 cm^2 sample from the envelope or stamp, and then cut it into smaller pieces. Transfer the pieces to a 1.5 ml microcentrifuge tube (not provided).

Note: Before cutting out the envelope or stamp sample, surface contamination can be reduced by using a swab moistened with distilled water.

Add 300 µl Buffer ATL and 20 µl Proteinase K, close the lid, and mix by pulse-vortexing for 10 s. Continue the procedure from step 2.

1c. Lysing hair roots:

Add 300 µl Buffer ATL, 20 µl Proteinase K, and 20 µl 1 M DTT to a 1.5 ml microcentrifuge tube (not provided).

Cut off a 0.5–1 cm piece starting from the hair bulb and transfer it to the 1.5 ml microcentrifuge tube. Close the lid and mix by pulse-vortexing for 10 s. Continue the procedure from step 2.

1d. Lysing hair shafts (without roots):

Add 300 µl Buffer ATL, 20 µl Proteinase K, and 20 µl 1 M DTT to a 1.5 ml microcentrifuge tube (not provided).

Cut the hair shaft into 0.5–1 cm pieces and transfer them to the 1.5 ml microcentrifuge tube. Close the lid and mix by pulse-vortexing for 10 s. Continue the procedure from step 2.

1e. Lysing nail clippings:

Transfer the nail clippings to a 1.5 ml microcentrifuge tube (not provided).

Add 300 µl Buffer ATL, 20 µl Proteinase K, and 20 µl 1 M DTT, close the lid, and mix by pulse-vortexing for 10 s. Continue the procedure from step 2.

1f. Lysing material stained with blood, saliva, or semen:

Cut out up to 0.5 cm^2 of stained material and then cut it into smaller pieces. Transfer the pieces to a 2 ml microcentrifuge tube (not provided).

Add 300 µl Buffer ATL and 20 µl Proteinase K. If processing semen stains, add 20 µl 1 M DTT as well. Close the lid and mix by pulse-vortexing for 10 s. Continue the procedure from step 2.

2. Place the tube in a thermomixer or heated orbital incubator, and incubate at 56°C with shaking at 900 rpm for at least 1 h.

In general, hairs are lysed in 1 h. If necessary, increase the incubation time to ensure complete lysis.

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

For larger samples of nail clippings, we recommend overnight incubation at 56°C. Any material that is not lysed during this incubation step or the incubation in step 5 will be pelleted during centrifugation in step 6.

3. **Briefly centrifuge the tube to remove drops from the inside of the lid.**
4. **Add 300 µl Buffer AL, close the lid, and mix by pulse-vortexing for 10 s.**

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

   A white precipitate may form when Buffer AL is added to Buffer ATL. The precipitate does not interfere with the QIAamp procedure and will dissolve during incubation in step 5.

   Note: If carrier RNA is required (see page 12), add 1 µg dissolved carrier RNA to 300 µl Buffer AL. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL.
5. **Place the tube in the thermomixer or heated orbital incubator, and incubate at 70°C with shaking at 900 rpm for 10 min.**

   If using a heating block or water bath, vortex the tube for 10 s every 3 min to improve lysis.
6. **Centrifuge the tube at full speed (20,000 x *g*; 14,000 rpm) for 1 min.**
7. **Carefully transfer the supernatant from step 6 to the QIAamp MinElute Column without wetting the rim.**

   Lysate remaining in solid sample material (e.g., denim) can be harvested by transferring the material to a QIAshredder Spin Column (not supplied) and centrifuging at full speed for 2 min. Transfer the flow-through to the QIAamp MinElute Column without wetting the rim.
8. **Close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

   If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the QIAamp MinElute Column is empty.
9. **Carefully open the QIAamp MinElute Column and add 500 µl Buffer AW1 without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

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

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

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

12. **Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute Column and apply 20–50 µl Buffer AE or distilled water to the center of the membrane.**

    If high pH or EDTA affects sensitive downstream applications, use water for elution (see page 11).

    Important: Ensure that Buffer AE or distilled water is equilibrated to room temperature (15–25°C). Dispense Buffer AE or distilled water onto the center of the membrane to ensure complete elution of bound DNA.

    QIAamp MinElute Columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. Remember that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.

13. **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 MinElute Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

Protocol: Isolation of Genomic DNA from Urine

This protocol is for isolation of genomic DNA from urine.

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Check whether carrier RNA is required (see pages 12 and 14).

Things to do before starting

- Equilibrate Buffer AE or distilled water for elution to room temperature (15–25°C).
- Set a thermomixer or heated orbital incubator to 56°C for use in step 5. If a thermomixer or heated orbital incubator is not available, a heating block or water bath can be used instead.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Optional: Prepare an aqueous 1 M DTT (dithiothreitol) stock solution. Store aliquots at –20°C. Thaw immediately before use.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 13.

Procedure

1. **Transfer up to 1 ml urine to a 1.5 ml microcentrifuge tube (not provided) and centrifuge at 6000 x *g* (8000 rpm) for 2 min.**

   If the volume of the urine sample is between 1 and 10 ml, pellet the cells in an appropriately sized centrifugation tube. Discard the supernatant, add 500 µl Buffer AE to the pellet, and vortex for 5 s. Briefly centrifuge and transfer the sample to a 1.5 ml microcentrifuge tube (not provided). Continue the procedure from step 3.

2. **Discard the supernatant, add 500 µl Buffer AE, and vortex for 5 s.**
3. **Centrifuge at 6000 x *g* (8000 rpm) for 2 min.**
4. **Discard the supernatant, add 300 µl Buffer ATL and 20 µl Proteinase K to the pellet, and mix by pulse-vortexing for 10 s.**

   Adding 20 µl 1 M DTT may increase sensitivity, since urine can contain sperm cells which can only be lysed in the presence of reducing agents such as DTT or β-mercaptoethanol.

5. **Place the 1.5 ml tube in a thermomixer or heated orbital incubator, and incubate at 56°C with shaking at 900 rpm for 1 h.**

   If using a heating block or water bath, vortex the tube for 10 s every 15 min to improve lysis.

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

7. **Add 300 µl Buffer AL and 50 µl ethanol (96–100%), close the lid, and mix by pulse-vortexing for 10 s.**

   To ensure efficient binding in step 9, it is essential that the sample, Buffer AL, and ethanol are thoroughly mixed to yield a homogeneous solution.

   Note: We recommend adding carrier RNA to Buffer AL (see page 14). Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL.

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

   Note: The white precipitate which may appear in step 7 does not need to be pelleted. It can be transferred to the QIAamp MinElute Column together with the lysate, and does not interfere with the QIAamp procedure.

9. **Carefully transfer the supernatant from step 8 to the QIAamp MinElute Column without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

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

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

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

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

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

13. **Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute Column and apply 20–50 µl Buffer AE or distilled water to the center of the membrane.**

    If high pH or EDTA affects sensitive downstream applications, use water for elution (see page 11).

    Important: Ensure that Buffer AE or distilled water is equilibrated to room temperature (15–25°C).Dispense Buffer AE or distilled water onto the center of the membrane to ensure complete elution of bound DNA.

    QIAamp MinElute Columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. 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 MinElute Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

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).
- If isolating DNA from very small amounts of tissue, carrier RNA is required (see pages 12 and 14).
- 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 ATL in step 2.

Things to do before starting

- Equilibrate Buffer AE or distilled water for elution to room temperature.
- Set a thermomixer or heated orbital incubator to 56°C for use in step 4. If a thermomixer or heated orbital incubator is not available, a heating block or water bath can be used instead.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 13.

Procedure

1. **Transfer a tissue sample of less than 10 mg in weight to a 1.5 ml microcentrifuge tube (not provided).**
2. **Immediately add 180 µl Buffer ATL, 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 or until the sample is completely lysed.**

   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 AL, close the lid, and mix by pulse-vortexing for 15 s.**

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

   Note: If carrier RNA is required (see page 12), add 1 µg dissolved carrier RNA to 200 µl Buffer AL. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AE and then added to Buffer AL.

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 the inside of the lid.**

8. **Carefully transfer the entire lysate from step 7 to the QIAamp MinElute Column without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

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

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

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

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

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

12. **Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute Column and apply 20–100 µl Buffer AE or distilled water to the center of the membrane.**

    If high pH or EDTA affects sensitive downstream applications, use water for elution (see page 11).

    Important: Ensure that Buffer AE or distilled water is equilibrated to room temperature (15–25°C). If using small elution volumes (<50 µl), dispense Buffer AE or distilled water onto the center of the membrane to ensure complete elution of bound DNA.

QIAamp MinElute Columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. Remember that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.

13. **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 MinElute Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

Protocol: Isolation of Genomic DNA from Laser-Microdissected Tissues

This protocol is for isolation of genomic DNA from laser-microdissected tissue. Laser-microdissected tissue specimens present a particular challenge for molecular analysis, as nucleic acids must be purified from very small amounts of starting material. In addition, fixation and staining steps may compromise the integrity of DNA, and it may be necessary either to modify fixation protocols or to use cryosections from flash-frozen specimens to minimize this problem.

A wide range of equipment and consumables for sectioning, staining, and microdissection of specimens is available from Leica (www.leica-microsystems.com).

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- If isolating DNA from very small numbers of cells, carrier RNA is required (see pages 12 and 14).

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Equilibrate Buffer AE or distilled water for elution to room temperature.
- Set a thermomixer or heated orbital incubator to 56°C for use in step 3. If a thermomixer or heated orbital incubator is not available, a heating block or water bath can be used instead.
- If Buffer AL or Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 13.

Procedure

1. **Add 15 µl Buffer ATL to a laser-microdissected sample collected in a 0.2 ml microcentrifuge tube (not provided).**
2. **Add 10 µl Proteinase K and mix by pulse-vortexing for 15 s.**
3. **Place the 0.2 ml tube in a thermomixer or heated orbital incubator, and incubate at 56°C for 3 h (16 h for formalin-fixed tissues) with occasional agitation.**

   The incubation time may vary depending on the amount of tissue collected.
4. **Add 25 µl Buffer ATL.**
5. **Add 50 µl Buffer AL, close the lid, and mix by pulse-vortexing for 15 s.**

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

6. **Add 50 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 0.2 ml tube to remove drops from the inside of the lid.**

8. **Carefully transfer the entire lysate from step 7 to the QIAamp MinElute Column without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

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

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

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

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

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

12. **Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute Column and apply 20–30 µl Buffer AE or distilled water to the center of the membrane.**

    If high pH or EDTA affects sensitive downstream applications, use water for elution (see page 11).

    Important: Ensure that Buffer AE or distilled water is equilibrated to room temperature (15–25°C). Dispense Buffer AE or distilled water onto the center of the membrane to ensure complete elution of bound DNA.

QIAamp MinElute Columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. Remember that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.

13. **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 MinElute Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

Protocol: Cleanup of Genomic DNA

This protocol is for cleanup of genomic DNA. Use this protocol to restore the suitability of the DNA for PCR, or to increase the concentration of the DNA.

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 AE or distilled water for elution to room temperature.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 13.

Procedure

1. **Add up to 100 µl of genomic DNA (containing up to 10 µg DNA) to a 1.5 ml microcentrifuge tube (not provided).**

   If the volume of DNA is less than 100 µl, add deionized water to a final volume of 100 µl.
2. **Add 10 µl Buffer AW1.**
3. **Add 250 µl Buffer AW2 and mix by pulse-vortexing for 10 s.**
4. **Transfer the entire sample from step 3 to the QIAamp MinElute Column without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**
5. **Carefully open the QIAamp MinElute Column and add 500 µl Buffer AW2 without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

   Contact between the QIAamp MinElute Column and the flow-through should be avoided. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains ethanol, coming into contact with the QIAamp MinElute Column. Take care when removing the QIAamp MinElute Column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp MinElute Column.
6. **Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min to dry the membrane completely.**

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

7. **Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute Column and apply 20–100 µl Buffer AE or distilled water to the center of the membrane.**

   If high pH or EDTA affects sensitive downstream applications, use water for elution (see page 11).

   Important: Ensure that Buffer AE or distilled water is equilibrated to room temperature (15–25°C). If using small elution volumes (<50 µl), dispense Buffer AE or distilled water onto the center of the membrane to ensure complete elution of bound DNA.

   QIAamp MinElute Columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. Remember that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.

8. **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 MinElute Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or molecular biology applications (see back page for contact information).

		Comments and suggestions
Little or no DNA in the eluate		
a)	Carrier RNA was not added to Buffer AL	Dissolve carrier RNA in Buffer AE and mix with Buffer AL as described on page 14. Repeat the purification procedure with new samples.
b)	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.
c)	Samples were kept at room temperature for too long	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, stains, or swabs can be stored at room temperature in the dark without significant DNA degradation.
d)	Insufficient sample lysis in Buffer AL	Proteinase K was stored at high temperatures for a prolonged time. Repeat the procedure using new samples and fresh Proteinase K.
e)	Buffer AL–carrier RNA mixture was mixed insufficiently	Mix Buffer AL with carrier RNA by gently inverting the tube at least 10 times.
f)	Low-percentage ethanol was used instead of 96–100%	Repeat the purification procedure with new samples and 96–100% ethanol.
g)	Buffer AW1 or AW2 was prepared incorrectly	Check that the Buffer AW1 and Buffer AW2 concentrates were diluted with the correct volume of 96–100% ethanol. Repeat the purification procedure with new samples, if available.
h)	pH of water used for elution was too low	DNA does not dissolve easily in acidic solutions. Ensure that the pH of the water used for elution is >7.0.

DNA does not perform well in downstream enzymatic reactions

a)	Little or no DNA in the eluate	See "Little or no DNA in the eluate" (page 43) for possible reasons. Increase the amount of eluate added to the reaction, if possible.
b)	Too much or too little carrier RNA in the eluate	Determine the maximum amount of carrier RNA suitable for your amplification reaction. Adjust the concentration of carrier RNA added to Buffer AL accordingly.
c)	Reduced sensitivity	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 water instead of Buffer AE for elution (see page 11).
d)	Performance of purified DNA in downstream assays varies with the age of the reconstituted wash buffers	Salt and ethanol components of wash Buffers AW1 and AW2 may have separated out after being unused for a long period. Always mix buffers thoroughly before each purification procedure.
e)	Eluate used for PCR contains too much Buffer AE	Buffer AE contains EDTA and is buffered at pH 9.0. Some *Taq* Polymerases are inhibited by EDTA and/or have a narrow pH optimum. Use distilled water for elution instead of Buffer AE. Use another DNA polymerase, such as QIAGEN HotStarTaq® DNA Polymerase.

General handling

a)	Clogged QIAamp MinElute Column	Incomplete lysis caused clogging of the membrane. Increase the lysis time to fully lyse the sample.
b)	Variable elution volumes	Different sample types have been processed.

Appendix: Working with DNA

General handling

Proper microbiological aseptic technique should always be used when working with small sample sizes. Hands and dust particles may carry bacteria and molds, and are the most common sources of contamination. Always wear latex or vinyl gloves while handling reagents and samples to prevent contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed.

Disposable plasticware

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

Ordering Information

Product	Contents	Cat. no.
QIAamp DNA Micro Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56304
Accessories		
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer	19076
Buffer AL (216 ml)	216 ml Lysis Buffer	19075
Buffer AW1 (concentrate, 242 ml)	242 ml Wash Buffer (1) Concentrate	19081
Buffer AW2 (concentrate, 324 ml)	324 ml Wash Buffer (2) Concentrate	19072
Buffer AE (240 ml)	240 ml Elution Buffer AE	19077
QIAGEN Proteinase K (2 ml)*	2 ml (>600 mAU/ml, solution)	19131
QIAshredder (50)*	50 disposable cell-lysate homogenizers for use in nucleic acid minipreps, caps	79654
Collection Tubes (2 ml)	1000 Collection Tubes (2 ml)	19201
Related products		
QIAamp DNA Blood Mini Kit — for isolation of DNA from blood and related body fluids		
QIAamp DNA Blood Mini Kit (50)*	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
QIAamp DNA Mini Kit — for isolation of DNA		
QIAamp DNA Mini Kit (50)*	For 50 DNA preps: 50 QIAamp Mini Spin Columns, Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304

* Larger kit sizes available; please inquire.

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