June 2012

QIAamp® DNA Investigator Handbook

For purification of total (genomic and mitochondrial) DNA from surface and buccal swabs, FTA® and Guthrie cards, body fluid stains, chewing gum, cigarette butts, nail clippings, hair, paper and similar materials, small volumes of blood or saliva, tissues, laser-microdissected specimens, bones, teeth, and sexual assault specimens

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents

QIAamp DNA Investigator Kit Catalog no. Number of preps	(50) 56504 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 ATE	20 ml
Carrier RNA (red cap)	310 µg
Proteinase K	1.25 ml
Selection Guide	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 5 for safety information.

† Contains sodium azide as a preservative.

Storage

QIAamp MinElute columns should be stored at 2–8°C upon arrival and are stable under these conditions for at least one year after delivery. 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 and are stable for at least one year after delivery.

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

Intended Use

The QIAamp DNA Investigator 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.

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.

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

Quality Control

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

Introduction

The QIAamp DNA Investigator 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 forensic and human-identity sample materials.

The procedure is designed to ensure that there is no sample-to-sample cross-contamination. After sample lysis, the simple QIAamp DNA Investigator procedure, which is highly suited for simultaneous processing of multiple samples, yields pure DNA in less than 40 minutes.

DNA is eluted in Buffer ATE 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 inhibitors.

Principle and procedure

The QIAamp DNA Investigator procedure consists of 4 steps (see flowchart, page 8):

- Lyse: sample is lysed under denaturing conditions with proteinase K
- Bind: DNA binds to the membrane and contaminants flow through
- Wash: residual contaminants are washed away
- Elute: pure, concentrated DNA is eluted from the membrane

Automated DNA purification on the QIAcube®

Purification of DNA from forensic and human-identity samples using the QIAamp DNA Investigator Kit can be 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). 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.

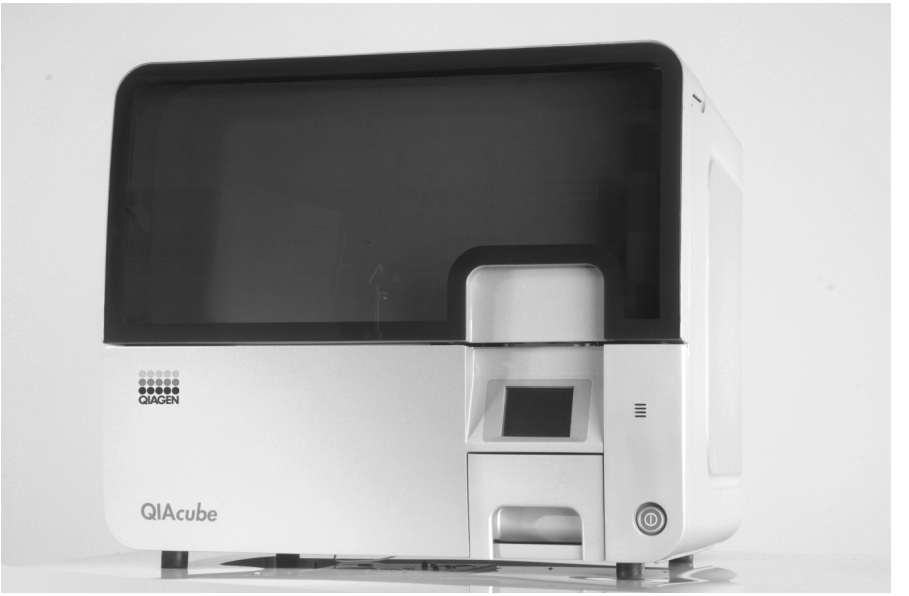
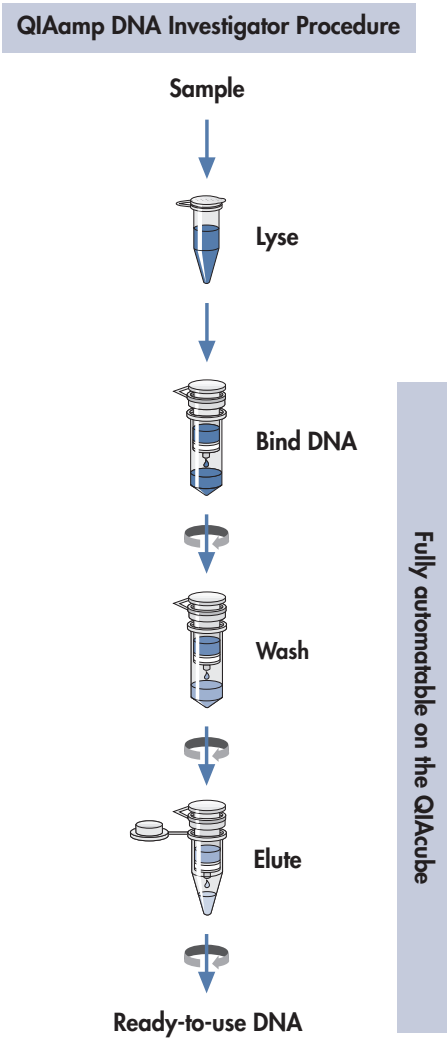


Figure 1. Automated DNA purification. DNA purification using the QIAamp DNA Investigator Kit can be automated on the QIAcube.


QIAamp DNA Investigator Procedure
Sample
Lyse
Bind DNA
Wash
Elute
Ready-to-use DNA
Fully automatable 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.

- Ethanol (96–100%)*
- 0.2 ml, 1.5 ml, or 2 ml microcentrifuge tubes (for lysis steps)
- 1.5 ml microcentrifuge tubes (for wash and 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)
- Thermomixer, heated orbital incubator, heating block, or water bath
- Microcentrifuge with rotor for 2 ml tubes

For swabs, FTA and Guthrie cards, chewing gum, cigarette butts, nail clippings and hair, paper, and similar materials

- Scissors or appropriate cutting device

For swabs and stained fabrics

- Optional: QIAshredder spin columns (for maximum yields), see page 55 for ordering information

For nail clippings and hair, semen stains, and sexual assault specimens

- Dithiothreitol (DTT), 1 M aqueous solution

For laser-microdissected specimens

- 0.2 ml microcentrifuge tubes (for lysis steps)

For bones and teeth

- Metal blender (e.g., Waring)† or TissueLyser II with the Grinding Jar Set, S. Steel, see page 55 for ordering information
- Liquid nitrogen

For sexual assault specimens

- Additional Buffer ATL, see page 55 for ordering information

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

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

Important Notes

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 Investigator 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 in 2 steps of 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 ATE 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 ATE 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 Investigator Kit

Protocol	Volume of Buffer AL added to sample (µl)	Dissolved carrier RNA (µl)
Surface and buccal swabs	600* or 400†	1
FTA and Guthrie cards	300	1
Body fluid stains	300	1
Chewing gum	300	1
Cigarette butts	300	1
Nail clippings and hair	300	1
Paper and similar materials	300	1
Small volumes of blood or saliva	100	1
Tissues	200	1
Laser-microdissected specimens	50	1
Bones and teeth	300	1
Sexual assault specimens	300	1

* If using ejectable swabs (e.g., Whatman® Omni Swabs).

† If using nonejectable swabs (e.g., cotton or Dacron® swabs).

Protocol: Isolation of Total DNA from Surface and Buccal Swabs

This protocol is for isolation of total (genomic and mitochondrial) DNA from surface swabs, 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 10 and 12).

Things to do before starting

- Equilibrate Buffer ATE 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 (optional) step 15, 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 11.
- 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 10), 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 ATE 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 (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 MinElute column back into the 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 spin column (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 (in a 2 ml collection tube) without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

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

Note: Up to 200 µl lysate remains in the swab. To harvest this remaining lysate, place the swab in a QIAshredder spin column (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 700 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. **Carefully open the QIAamp 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 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 dry the membrane completely.**

    This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
15. **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 incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.**
16. **Apply 20–100 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. Dispense Buffer ATE 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. 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.
17. **Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.**

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

Protocol: Isolation of Total DNA from FTA and Guthrie Cards

This protocol is for isolation of total (genomic and mitochondrial) DNA from whole blood, saliva, or buccal cells dried and immobilized on FTA cards, Guthrie cards, or similar collection devices.

Important point before starting

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

Things to do before starting

- Equilibrate Buffer ATE 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 (optional) step 16, 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 11.
- Optional: If processing very small amounts of starting material, add carrier RNA dissolved in Buffer ATE to Buffer AL according to the instructions on page 12.

Procedure

1. **Cut 3 mm (1/8 inch) diameter punches from a dried spot with a single-hole paper punch. Place up to 3 card punches into a 1.5 ml microcentrifuge tube (not provided).**
2. **Add 280 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 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 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 12). Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer ATE 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. **Add 150 µl ethanol (96–100%), close the lid, and mix thoroughly by pulse-vortexing for 15 s.**

   To ensure efficient binding in step 11, it is essential that the sample and ethanol are thoroughly mixed to yield a homogeneous solution.

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

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

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

12. **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.**

13. **Carefully open the QIAamp MinElute column and add 700 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.

14. **Carefully open the QIAamp 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 MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

15. **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.

16. **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 incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.**

17. **Apply 20–100 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. If using small elution volumes (<50 µl), dispense Buffer ATE 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. 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.**

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

Protocol: Isolation of Total DNA from Body Fluid Stains

This protocol is for isolation of total (genomic and mitochondrial) DNA from material stained with blood, saliva, or semen.

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Check whether carrier RNA is required (see pages 10 and 12).

Things to do before starting

- Equilibrate Buffer ATE 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 (optional) step 16, 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.
- If processing semen stains, 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 11.
- Optional: If processing stained fabrics, QIAshredder spin columns may be required.

Procedure

1. **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).**
2. **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.**
3. **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.**

   If using a heating block or water bath, vortex the tube for 10 s every 10 min to improve lysis.
4. **Briefly centrifuge the 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 10), 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 ATE and then added to Buffer AL.

6. **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.

7. **Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.**

   If solid particles are still visible, centrifuge at 6000 x *g* (8000 rpm) for 1 min, and carefully transfer supernatant to a new 1.5 ml microcentrifuge tube (not provided).

   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 a new 1.5 ml microcentrifuge tube (not provided).

8. **Add 150 µl ethanol (96–100%), close the lid, and mix thoroughly 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 tube to remove drops from the inside of the lid.**

10. **Carefully transfer the supernatant from step 9 to the QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim.**

11. **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.

12. **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.**

13. **Carefully open the QIAamp MinElute column and add 700 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.

14. **Carefully open the QIAamp 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 MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

15. **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.

16. **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 incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.**

17. **Apply 20–50 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. Dispense Buffer ATE 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. 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.**

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

Protocol: Isolation of Total DNA from Chewing Gum

This protocol is for isolation of total (genomic and mitochondrial) 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 10 and 12).

Things to do before starting

- Equilibrate Buffer ATE 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 (optional) step 15, 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 11.

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 10), 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 ATE 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.**

   If solid particles are still visible, centrifuge at 6000 x *g* (8000 rpm) for 1 min, and carefully transfer supernatant to a new 1.5 ml microcentrifuge tube (not provided).

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 (in a 2 ml collection tube) without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

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 700 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. **Carefully open the QIAamp 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 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 dry the membrane completely.**

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

15. **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 incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.**

16. **Apply 20–50 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. Dispense Buffer ATE 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. 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.

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

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

Protocol: Isolation of Total DNA from Cigarette Butts

This protocol is for isolation of total (genomic and mitochondrial) DNA from cigarette butts.

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Check whether carrier RNA is required (see pages 10 and 12).

Things to do before starting

- Equilibrate Buffer ATE 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 (optional) step 16, 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 11.

Procedure

1. **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).**
2. **Add 300 µl Buffer ATL and 20 µl proteinase K, close the lid, and mix by pulse-vortexing for 10 s.**
3. **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.**

   If using a heating block or water bath, vortex the tube for 10 s every 10 min to improve lysis.
4. **Briefly centrifuge the 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 10), 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 ATE and then added to Buffer AL.

6. **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.

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

   If solid particles are still visible, centrifuge at 6000 x *g* (8000 rpm) for 1 min, and carefully transfer supernatant to a new 1.5 ml microcentrifuge tube (not provided).

8. **Add 150 µl ethanol (96–100%), close the lid, and mix thoroughly 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 1.5 ml tube to remove drops from the inside of the lid.**

10. **Carefully transfer the supernatant from step 9 to the QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim.**

11. **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.

12. **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.**

13. **Carefully open the QIAamp MinElute column and add 700 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.

14. **Carefully open the QIAamp 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 MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

15. **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.

16. **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 incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.**

17. **Apply 20–50 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. Dispense Buffer ATE 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. 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.**

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

Protocol: Isolation of Total DNA from Nail Clippings and Hair

This protocol is for isolation of total (genomic and mitochondrial) DNA from nail clippings and hair roots or shafts.

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Check whether carrier RNA is required (see pages 10 and 12).

Things to do before starting

- Equilibrate Buffer ATE 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 (optional) step 15, 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.
- 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 11.

Procedure

1. Lyse the samples according to step 1a for nail clippings, step 1b for hair roots, or step 1c for hair shafts (without roots).

1a. 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.

1b. 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.

1c. 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 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.

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 10), 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 ATE 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. **Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.**

7. **Add 150 µl ethanol (96–100%), close the lid, and mix thoroughly by pulse-vortexing for 15 s.**

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

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

9. **Carefully transfer the supernatant from step 8 to the QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim.**

10. **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 700 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. **Carefully open the QIAamp 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 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 dry the membrane completely.**

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

15. **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 incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.**

16. **Apply 20–50 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. Dispense Buffer ATE 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. 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.

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

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

Protocol: Isolation of Total DNA from Paper and Similar Materials

This protocol is for isolation of total (genomic and mitochondrial) DNA from paper evidence samples, such as saliva on envelope flaps and stamps or fingerprints on documents.

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Check whether carrier RNA is required (see pages 10 and 12).

Things to do before starting

- Equilibrate Buffer ATE 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 (optional) step 16, 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 11.

Procedure

1. **Cut out a 0.5–2.5 cm² sample from the paper or similar material, and then cut it into smaller pieces. Transfer the pieces to a 1.5 ml microcentrifuge tube (not provided).**

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

2. **Add 300 µl Buffer ATL and 20 µl proteinase K, close the lid, and mix by pulse-vortexing for 10 s.**

3. **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.**

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

4. **Briefly centrifuge the 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 10), 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 ATE and then added to Buffer AL.

6. **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.

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

   If solid particles are still visible, centrifuge at 6000 x *g* (8000 rpm) for 1 min, and carefully transfer supernatant to a new 1.5 ml microcentrifuge tube (not provided).

8. **Add 150 µl ethanol (96–100%), close the lid, and mix thoroughly 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 1.5 ml tube to remove drops from the inside of the lid.**

10. **Carefully transfer the supernatant from step 9 to the QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim.**

11. **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.

12. **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.**

13. **Carefully open the QIAamp MinElute column and add 700 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.

14. **Carefully open the QIAamp 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 MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

15. **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.

16. **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 incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.**

17. **Apply 20–50 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. Dispense Buffer ATE 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. 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.**

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

Protocol: Isolation of Total DNA from Small Volumes of Blood or Saliva

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

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 ATE or distilled water for elution to room temperature.
- Set a thermomixer or heated orbital incubator to 56°C for use in step 5 and (optional) step 14.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 11.
- 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 ATE to Buffer AL according to the instructions on page 12.

Procedure

1. **Pipet 1–100 µl whole blood or saliva 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 10). Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer ATE 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 (15–25°C).**

   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 (in a 2 ml collection tube) without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

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 700 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. **Carefully open the QIAamp 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 MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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 incubate at room temperature for 10 min or at 56°C for 3 min.**

15. **Apply 20–100 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. If using small elution volumes (<50 µl), dispense Buffer ATE 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. 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.

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

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

Protocol: Isolation of Total DNA from Tissues

This protocol is for isolation of total (genomic and mitochondrial) 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 10 and 12).
- 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 ATE 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 4 and (optional) step 13. 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 11.

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 10), 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 ATE 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.**

   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 (in a 2 ml collection tube) without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

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 700 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. **Carefully open the QIAamp 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 MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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 incubate at room temperature for 10 min or at 56°C for 3 min.**

14. **Apply 20–100 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. If using small elution volumes (<50 µl), dispense Buffer ATE 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. 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.

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

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

Protocol: Isolation of Total DNA from Laser-Microdissected Specimens

This protocol is for isolation of total (genomic and mitochondrial) 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 10 and 12).

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Equilibrate Buffer ATE or distilled water for elution to room temperature.
- Set a thermomixer or heated orbital incubator to 56°C for use in step 3 and (optional) step 13. 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 11.

Procedure

1. **Add 15 µl Buffer ATL to a laser-microdissected sample collected in a 0.2 ml micro-centrifuge 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.

   Note: If carrier RNA is required (see page 10), add 1 µg dissolved carrier RNA to 50 µl Buffer AL. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer ATE and then added to Buffer AL.

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 (in a 2 ml collection tube) without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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

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 700 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. **Carefully open the QIAamp 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 MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**

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 incubate at room temperature for 10 min or at 56°C for 3 min.**

14. **Apply 20–30 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. Dispense Buffer ATE 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. 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.

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

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

Protocol: Isolation of Total DNA from Bones and Teeth

This protocol is for isolation of total (genomic and mitochondrial) DNA from pieces of bones and teeth.

Important points before starting

- Lysis time will vary depending on the size and density of the source material. The lysis conditions given here are intended to serve as guidelines.
- Perform all centrifugation steps at room temperature (15–25°C).

Things to do before starting

- Equilibrate Buffer ATE 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 (optional) step 15, 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.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 11.
- Optional: If processing very small amounts of starting material, add carrier RNA dissolved in Buffer ATE to Buffer AL according to the instructions on page 12.

Procedure

1. **Crush the bone into small fragments. Grind to a fine powder using a metal blender half-filled with liquid nitrogen. Alternatively, grind the bone to a fine powder using the TissueLyser II and the Grinding Jar Set, S. Steel.**

   When using the TissueLyser II, transfer the bone sample and the ball into the grinding jar. Pour liquid nitrogen into the grinding jar over the ball and bone fragments. Allow the temperature to equilibrate (i.e., liquid nitrogen stops boiling). Decant the excess liquid nitrogen, close the grinding jar with the lid, and transfer it to the TissueLyser II. Grind the bone at 30 Hz for 1 min or until the bone is pulverized (grinding times depend on type, condition, and size of bone).

2. **Place ≤100 mg of powdered bone into a 1.5 ml microcentrifuge tube. Add 360 µl Buffer ATL and 20 µl proteinase K. Incubate overnight at 56°C.**

   After incubation, set the temperature to 70°C for the incubation in step 5.

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 10), 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 ATE 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, and carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube (not provided).**

7. **Add 150 µl ethanol (96–100%). Close the lid, and mix by pulse-vortexing for 15 s.**

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

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 (in a 2 ml collection tube) without wetting the rim.**

10. **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 600 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 700 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. **Carefully open the QIAamp 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 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 dry the membrane completely.**

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

15. **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 incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.**

16. **Apply 20–50 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. Dispense Buffer ATE 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. 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.

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

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

Protocol: Isolation of Total DNA from Sexual Assault Specimens

This protocol is for differential extraction of total (genomic and mitochondrial) DNA from fabrics or swabs containing epithelial cells mixed with sperm cells.

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).
- Additional Buffer ATL is required (see page 55 for ordering information).

Things to do before starting

- Equilibrate Buffer ATE or distilled water for elution to room temperature (15–25°C).
- Set a thermomixer or heated orbital incubator to 56°C for use in steps 3, 10, and (optional) 23, and a second thermomixer or heated orbital incubator to 70°C for use in step 13. 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 11.
- Prepare an aqueous 1 M DTT (dithiothreitol) stock solution. Store aliquots at –20°C. Thaw immediately before use.
- Optional: If processing very small amounts of starting material, add carrier RNA dissolved in Buffer ATE to Buffer AL according to the instructions on page 12.

Procedure

1. **Place the swab or a piece of fabrics (≤0.5 cm²) in a 2 ml microcentrifuge tube (not provided).**

   Separate the cotton or DACRON swab from its shaft by hand or using scissors.
2. **Add 20 µl proteinase K and 500 µl Buffer ATL to the sample. Close the cap 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. **Remove the solid material from the tube.**

   Note: Up to 200 µl of lysate remains in the swab or fabric. To harvest this remaining lysate, place the swab or fabric in a QIAshredder spin column (not supplied), place the QIAshredder spin column containing the solid material in the 2 ml tube containing the lysate, and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 2 min. Remove and discard the QIAshredder spin column containing the solid material.

6. **Centrifuge the tube for 5 min at full speed. Carefully transfer all but 30 µl of the supernatant to a new tube without disturbing the pellet.**

   Note: For isolation of DNA from epithelial cells, transfer 300 µl of the supernatant into a 2 ml microcentrifuge tube and continue with step 12.

7. **Resuspend the pellet in 500 µl Buffer ATL. Close the lid and mix by pulse-vortexing for 10 s. Centrifuge the tube for 5 min at full speed. Carefully aspirate and discard all but 30 µl of the supernatant without disturbing the pellet.**

8. **Repeat step 7 at least three times.**

   Note: The ratio of epithelial cells to sperm cells influences the number of repeats needed for purification of sperm nuclei.

9. **Add 280 µl Buffer ATL, 10 µl proteinase K, and 10 µl 1 M DTT to the pellet. Close the lid and mix by pulse-vortexing for 10 s.**

10. **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 hour.**

    If using a thermoblock or waterbath, vortex the tube for 10 s every 10 min to improve lysis.

11. **Briefly centrifuge the tube to remove drops from the inside of the lid.**

12. **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 13.

    Note: If carrier RNA is required (see page 10), 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 ATE and then added to Buffer AL.

13. **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.

14. **Centrifuge the tube at full speed (20,000 x *g*; 14,000 rpm) for 1 min, and carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube (not provided).**
15. **Add 150 µl ethanol (96–100%). Close the lid, and mix by pulse-vortexing for 15 s.**

    To ensure efficient binding in step 17, it is essential that the sample and ethanol are thoroughly mixed to yield a homogeneous solution.
16. **Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.**
17. **Carefully transfer the entire lysate from step 16 to the QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim.**
18. **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.
19. **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.**
20. **Carefully open the QIAamp MinElute column and add 700 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.
21. **Carefully open the QIAamp 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 MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.**
22. **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.
23. **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 incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.**

24. **Apply 20–50 µl Buffer ATE or distilled water to the center of the membrane.**

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. Dispense Buffer ATE 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. 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.

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

    Incubating the QIAamp MinElute column loaded with Buffer ATE 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. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
Little or no DNA in the eluate		
a)	Carrier RNA was not added to to Buffer AL	Dissolve carrier RNA in Buffer ATE and mix with Buffer AL as described on page 12. 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 (15–25°C). Where possible, always use fresh samples, or store the samples at 2–8°C (nondried 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. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

		Comments and suggestions
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 51) 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.
d)	Performance of purified DNA in in downstream assay 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.
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 A: 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.

Appendix B: Cleanup of DNA

This protocol is for cleanup of 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 ATE or distilled water for elution to room temperature.
- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on page 11.

Procedure

B1. Add up to 100 µl of 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.

B2. Add 10 µl Buffer AW1.

B3. Add 250 µl Buffer AW2 and mix by pulse-vortexing for 10 s.

B4. Transfer the entire sample from step 3 to the QIAamp MinElute column (in a 2 ml collection tube) without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

B5. 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.

B6. 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.

B7. Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (not provided) and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute column and apply 20–100 µl Buffer ATE or distilled water to the center of the membrane.

Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature (15–25°C). If using small elution volumes (<50 µl), dispense Buffer ATE 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. 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.

B8. Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.

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

Ordering Information

Product	Contents	Cat. no.
QIAamp DNA Investigator Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56504
QIAcube — for fully automated sample preparation using QIAGEN spin-column kits		
QIAcube (110 V)*† QIAcube (230 V)‡	Robotic workstation for automated purification of nucleic acids or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor§	9001292*† 9001293‡
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
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
TissueLyser II	Bead mill, 100-120/220-240 V, 50/60 Hz; requires the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 (available separately)	85300
Grinding Jar Set, S. Steel (2 x 10 ml)	2 Grinding Jars (10 ml), 2 Stainless Steel Grinding Balls (20 mm)	69985

* US and Canada.

† Japan.

‡ Rest of world.

§ Agreements for comprehensive service coverage are available; please inquire.

¶ Larger kit sizes available; please inquire.

Ordering Information

Product	Contents	Cat. no.
Related products		
EZ1® DNA Investigator Kit — for easy, automated purification of DNA from a wide variety of forensic and human-identity samples		
EZ1 DNA Investigator Kit (48)	For 48 preps on EZ1 workstations: Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers and Reagents; includes carrier RNA	952034
EZ1 DNA Investigator Card	Preprogrammed card for EZ1 DNA Investigator protocols	9016387
MagAttract® DNA Mini M48 Kit — for automated purification of DNA from a wide range of human samples for forensic applications		
MagAttract DNA Mini M48 Kit (192)	For 192 DNA preps on the BioRobot® M48 workstation: MagAttract Suspension B, Buffers, Proteinase K	953336
App. Package, M48, Forensics	Software protocol package for forensics applications, v2.1, on the BioRobot M48 workstation	9016150
QIAamp 96 DNA Swab BioRobot Kit — for automated high-throughput DNA purification from swabs using the BioRobot Universal System		
QIAamp 96 DNA Swab BioRobot Kit (12)	For 12 x 96 DNA preps: 12 QIAamp 96 Plates, Buffers, QIAGEN Proteinase K, AirPore Tape Sheets, Tape Pad, S-Blocks, Racks with Collection Microtubes (1.2 ml), Caps	965842
QIAamp DNA Stool Mini Kit — for purification of up to 30 µg genomic, bacterial, viral, and parasite DNA from stool		
QIAamp DNA Stool Mini Kit (50)*	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, InhibitEX® tablets, Buffers, Collection Tubes (2 ml)	51504

* Fully automatable on the QIAcube. See www.qiagen.com/MyQIAcube for protocols.

Ordering Information

Product	Contents	Cat. no.
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
QIAamp DNA Micro Kit — for purification of genomic and mitochondrial DNA from small samples		
QIAamp DNA Micro Kit (50)*	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56304
QIAamp DNA FFPE Tissue Kit — For purification of DNA from formalin-fixed, paraffin-embedded tissues		
QIAamp DNA FFPE Tissue Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404

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* Fully automatable on the QIAcube. See www.qiagen.com/MyQIAcube for protocols.

† Larger kit sizes available; please inquire.

Notes

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1072908 06/2012