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MagAttract[®] DNA Mini M48 Forensic Handbook

MagAttract DNA Mini M48 Kit and the
App. Package, M48, Forensics v2.1 or v2.0

For automated purification of DNA from
forensic and biosecurity samples using the
BioRobot[®] M48 workstation



WWW.QIAGEN.COM

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Kit Contents

MagAttract DNA Mini M48 Kit	(192)
Catalog no.	953336
Number of preps	192
MagAttract Suspension B	2 x 10 ml
Buffer MTL	3 x 54 ml
Buffer MW1	2 x 77 ml
Buffer MW2	1 x 87 ml
RNase-Free Water	1 x 90 ml
Buffer G2	1 x 60 ml
QIAGEN® Proteinase K	1 x 2.0 ml
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App. Package, M48, Forensics v2.1	
Catalog no.	9016150
CD	1

Additional Buffer G2, Buffer MTL, and QIAGEN Proteinase K, required for some protocols, are available separately. See page 54 for ordering information.

Storage

All buffers and reagents should be stored at room temperature (15–25°C).

The ready-to-use QIAGEN Proteinase K solution is stable for up to one year after delivery when stored at room temperature. To prolong the lifetime of QIAGEN Proteinase K, storage at 2–8°C is recommended.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of MagAttract DNA Mini M48 Kits is tested against predetermined specifications to ensure consistent product quality.

Product Warranty and Satisfaction Guarantee

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

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

Product Use Limitations

The MagAttract DNA Mini M48 Kit is intended for molecular biology applications in forensic and biosecurity testing. 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.

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the MagAttract DNA Mini M48 Kit or QIAGEN products in general, please do not hesitate to contact us.

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

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

Safety Information

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

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

Buffers MW1 and MTL contain guanidine hydrochloride/guanidine thiocyanate, 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. If liquid containing potentially infectious agents is spilt on the BioRobot M48, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

The following risk and safety phrases apply to the components of the MagAttract DNA Mini M48 Kit:

QIAGEN Proteinase K

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

Buffer MTL

Contains guanidine thiocyanate: harmful. Risk and safety phrases:* R20/21/22-32, S13-26-36-46

Buffer MW1

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

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

* R20/21/22: Harmful by inhalation, in contact with skin, and if swallowed; R22: Harmful if swallowed; R32: Contact with acids liberates very toxic gas; R36/38: Irritating to eyes and skin; R36/37/38: Irritating to eyes, respiratory system, and skin; R42/43: May cause sensitization by inhalation and skin contact. S13: Keep away from food, drink, and animal feedingstuffs; S23: Do not breathe spray; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37: Wear suitable protective clothing and gloves; S46: If swallowed, seek medical advice immediately and show container or label.

Introduction

The BioRobot M48 and the MagAttract DNA Mini M48 Kit, with protocols on the App. Package, M48, Forensics, reproducibly automate purification of genomic DNA from 6–48 samples encountered in forensic and biosecurity applications. Purification is efficient and purified DNA performs well in downstream analyses, such as quantitative PCR and STR analysis, with high signal-to-noise ratios.

The BioRobot M48 workstation performs all steps of the sample preparation procedure, and the procedure can be scaled up or down, allowing purification from varying amounts of starting material. Up to 48 samples are processed in a single run.

Principle and procedure

Magnetic-particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (see flowchart, page 10). DNA is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted in the user's choice of either water or TE buffer. The user can choose elution volumes of 50–400 μl (50, 75, 100, 150, 200, 250, 300, or 400 μl).

MagAttract DNA Mini M48 Forensic Procedure

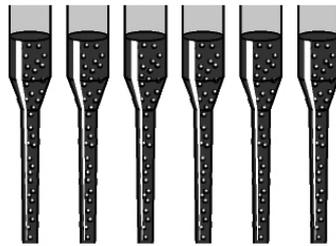
Blood or pretreated sample



Lysis



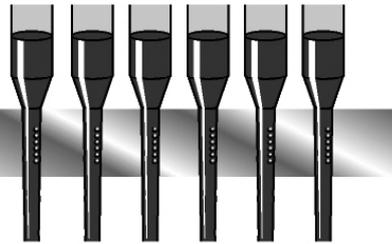
Magnetic particles added to samples



DNA binds to magnetic particles



Magnet

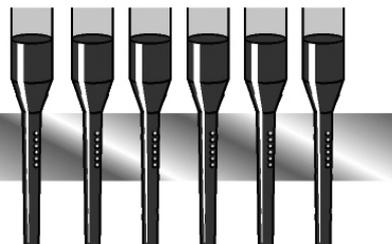


Magnetic separation



Wash

Magnet



Magnetic separation



Elute



Pure, high-quality DNA

Description of protocols

This handbook contains two types of protocols.

- Pretreatment protocols detail the preliminary steps, such as proteinase K digestion, prior to processing on the BioRobot M48 workstation.
- DNA purification protocols describe setting up the BioRobot M48 and starting a fully automated run.

Pretreatment protocols

Since the type of samples that can be processed using the MagAttract DNA Mini M48 Kit can vary greatly, there are also a variety of different pretreatments, optimized for specific sample types. For sample types not specifically included in this handbook, the Protocol: Pretreatment for Other Forensic Samples, page 39, provides a generic protocol that can serve as a starting point for optimizing pretreatment for other sample types.

DNA purification protocols

There are 3 DNA purification protocols, which can be used in conjunction with the pretreatment protocols. Within each protocol, the user can specify elution volumes of 50, 75, 100, 150, 200, 250, 300, or 400 μl . The standard **Protocol: DNA Purification (Trace Sample v3.1 or v3.0)**, page 41, can be used with all sample types.

In the **Protocol: DNA Purification (Trace TD “Tip Dance”)**, page 43, the filter-tip moves back-and-forth relative to the worktable platform while pipetting. This enables processing of solid materials, such as swabs, fabrics, blood discs, or cigarette butts, directly in the sample tube. There is generally no need for prior centrifugation to remove solid materials that could clog the tip. The “tip dance” protocol simplifies handling and, in some cases, increases DNA yield with more efficient extraction of the sample.

The **Protocol: DNA Purification (Large Volume)**, page 45, enables fully automated processing of starting volumes up to 500 μl . This not only allows efficient DNA purification from dilute samples with low concentrations of DNA, such as diffuse stains, but also enables purification from samples that require larger volumes for thorough lysis. The ability to process larger sample volumes — with the same elution volume as the standard trace protocol — enables higher yields of more concentrated DNA for greater sensitivity in downstream applications.

Sample-to-sample variability can result in widely varying yields from reference blood or saliva samples. Using the **normalization protocol** in Appendix B, page 51, DNA yields can be limited uniformly to 150–250 ng. This enables subsequent genetic analysis without the need to measure or adjust DNA concentration. The purified DNA is ready to use in downstream applications. This protocol has not been thoroughly tested and optimized by QIAGEN.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- BioRobot M48 workstation, cat. no. 9000708
- MagAttract DNA Mini M48 Kit, cat. no. 953336
- App. Package, M48, Forensics v2.1 or v2.0, cat. no. 9016150
- Filter-Tips, 1000 μ l, M48 (1000), cat. no. 995652
- Reagent Containers, small, M48 (100), cat. no. 995902
- Reagent Containers, large, M48 (50), cat. no. 995904
- Reagent Container Seals, M48 (50), cat. no. 995906
- Sample Prep Plates, 42-well, M48 (100), cat. no. 995908
- Sample tubes with screw caps, 1.5 ml (Sarstedt, cat. no. 72.692)* or 2 ml (Sarstedt, cat. no. 72.693)*
- Elution tubes with screw caps, 1.5 ml (Sarstedt, cat. no. 72.692)*[†] or 2 ml (Sarstedt, cat. no. 72.693)*[†]
- Centrifuge for 1.5 ml sample tubes, capable of centrifugation at 300 x g
- Thermomixer, heating block, or water bath
- Vortexer
- Pipets and pipet tips (to prevent cross-contamination, we strongly recommend the use of pipets tips with aerosol barriers)
- Ethanol (96–100%)[‡]
- Distilled water
- Optional: Sterile TE buffer (10 mM Tris·Cl, 0.1 mM EDTA, pH 8.3) for elution
- Optional: Cooling Block, 48-tube, 0.2 ml, M48, cat. no. 9015178
- Optional: Cooling Block, 48-tube, 1.4 ml, M48, cat. no. 9015180

* This is not a complete list of suppliers and does not include many important vendors of biological supplies; however, use of other tubes may result in an instrument crash.

[†] DNA can also be eluted into 0.2 ml thin-walled PCR tubes or 1.4 ml tubes.

[‡] Do not use denatured ethanol, which contains other substances such as methanol or methylethylketone.

For purification of DNA from dried blood

- Filter paper (e.g., 903[®] Specimen Collection Paper [Schleicher & Schuell, cat. no. 10538414], 903 Generic Blood Collection Card [Schleicher & Schuell, cat. no. 10538019], FTA[®] Classic Card [Whatman, cat. no. WB120205], FTA Indicating Classic Card [Whatman, cat. no. WB120206])*
- Manual paper punch, 3 mm (Schleicher & Schuell, cat. no. 10495010) or, alternatively, DBS puncher (PerkinElmer, cat. no. 1296-071)*

For purification of DNA from forensic surface and contact swabs

- Plastic swabs with cotton or Dacron[®] tips (Puritan[®] applicators with plastic shafts and cotton or Dacron tips are available from: Hardwood Products Company, www.hwppuritan.com, item nos. 25-806 1PC and 25-806 1PD; and from Daigger, www.daigger.com, cat. nos. EF22008D and EF22008DA). Nylon cytology brushes and other swab types may also be used.*

For purification of DNA from chewing gum

- Forceps

For purification of DNA from human tissues

- 1.5 ml screw-capped tubes

For purification of DNA from epithelial cells mixed with sperm cells

- Buffer G2, cat. no. 1014636
- 1 M dithiothreitol (DTT)
- Microcentrifuge
- Forceps

For purification of DNA from hair

- QIAGEN Proteinase K, cat. no. 19131 or 19133
- DTT solution (1 M dithiothreitol, 10 mM sodium acetate, pH 5.2)

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

For purification of DNA from bones or teeth

- QIAGEN Proteinase K, cat. no. 19131 or 19133
- 0.5 M EDTA, pH 8.3
- Liquid nitrogen
- 2 ml microcentrifuge tubes
- Microcentrifuge
- TissueLyser, cat. no. 85210 (North America), cat. no. 85200 (Japan), or 85220 (rest of world), with the Grinding Jar Set, S. Steel, cat. no. 69985; or an equivalent bead mill

For purification of DNA from soil

- InhibitEX[®] tablets (contact QIAGEN Technical Services, see back cover)
- Microcentrifuge

For DNA purification using the large-volume protocol

- Buffer MTL, cat. no. 1023430
- Sample tubes with screw caps, 2 ml (Sarstedt, cat. no. 72.693)*

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

Important Notes

Starting material

The amount of starting material for use in MagAttract DNA Mini M48 forensic procedures can vary greatly, depending on the amount of DNA in the sample. Specific guidance for starting amounts are given in the individual protocols. The BioRobot M48 can process 200 μ l pretreated samples using the trace protocol (page 41) or the “tip dance” protocol (page 43) for DNA purification. With the large-volume protocol (page 45), up to 500 μ l pretreated samples can be processed.

Lysis with proteinase K

The MagAttract DNA Mini M48 Kit contains proteinase K, which is the enzyme of choice for lysis buffers used in the MagAttract DNA Mini M48 forensic protocols. Proteinase K is particularly suitable for short digestion times. It possesses a high specific activity and remains stable over a wide range of temperatures and pH values, with substantially increased activity at higher temperatures. The activity of the proteinase K solution is 600 mAU/ml solution (or 40 mAU/mg protein). This activity provides optimal results in MagAttract DNA Mini M48 forensic protocols.

Additional QIAGEN Proteinase K is required for purification of DNA from hair, bones, or teeth (see page 54 for ordering information).

Buffer MTL

Before use, check that Buffer MTL does not contain a white precipitate by shaking the bottle. Check again when pipetting Buffer MTL into the reagent container. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Between runs, store Buffer MTL at room temperature (15–25°C).

Buffer MW1

Add 26 ml of ethanol (96–100%) to a bottle containing 77 ml of Buffer MW1 (provided) as described on the bottle. Do not use denatured ethanol, which contains other substances such as methanol or methylethylketone. Tick the check box on the bottle to indicate that ethanol has been added. Between runs, store the reconstituted Buffer MW1 at room temperature (15–25°C).

Note: Always mix Buffer MW1 by shaking the bottle five times before starting the procedure.

Buffer MW2

Buffer MW2 should not be used in MagAttract DNA Mini M48 forensic protocols. When prompted to add Buffer MW2 (in protocols on the App. Package, M48, Forensics v2.0 only), use distilled water instead. Distilled water provides more efficient removal of PCR inhibitors during the wash steps of MagAttract DNA Mini M48 forensic protocols.

MagAttract Suspension B

Shake the bottle containing MagAttract Suspension B and vortex for 3 minutes (before first use) or 1 minute (before subsequent uses) to ensure that the magnetic silica particles are fully resuspended.

Residual reagents

Residual reagents should either be removed immediately from the workstation and transferred to an airtight container, or discarded. Residual Buffer MTL should always be discarded.

Setting up the worktable

The worktable of the BioRobot M48 is where the user loads samples, plasticware, and the components of the MagAttract DNA Mini M48 Kit.

Details on worktable setup are summarized in Table 1 on page 17 and are also displayed in the protocol messages when the user starts worktable setup.

Quantification of DNA

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified DNA but should not affect downstream applications. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. See "Quantification of DNA", page 49, for more information.

Generally, there is very little carryover of magnetic particles due to the small number of particles required for forensic samples. If necessary, to eliminate carried-over magnetic particles, the tube containing the eluate can first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see "Quantification of DNA", page 49).

Table 1. Summary of Worktable Setup for MagAttract DNA Mini M48 Forensic Procedures

Item	Position
Filter-tips	Tip racks
Buffer MTL	Large reagent container 1
Buffer MW1	Large reagent container 2
Ethanol (96–100%)	Large reagent container 3
Distilled water	Large reagent container 4
MagAttract Suspension B	Small reagent container 1
RNase-free water or TE buffer	Small reagent container 2
Distilled water*	Small reagent container 3
Assay plates	Assay plate positions
Sample tubes (1.5 ml), open	Heating block
Elution tubes (1.5 ml or 2 ml), open	Elution block
Pretreated sample in 1.5 ml or 2 ml sample tubes, open	Sample rack

* Use distilled water instead of Buffer MW2.

Yield of purified DNA

DNA yields depend on the sample type, number of nucleated cells in the sample, and the protocol used for DNA purification. The maximum possible yield using MagAttract DNA Mini M48 forensic procedures is approximately 2 µg. Table 2 shows typical yields for some common reference sample types.

Table 2. DNA Yields from Common Reference Sample Types Using MagAttract DNA Mini M48 Forensic Procedures

Sample type	Sample amount	Protocol	DNA yield
Blood*	10–200 µl	Trace or Tip dance	150 ng – 2 µg
Blood*	10–200 µl	Normalization	150–250 ng
Dried blood	4 x 3 mm discs	Tip dance	0.2–0.5 µg
Buccal cells	1 swab	Tip dance	100 ng – 2 µg

* Whole blood with 3–7 x 10⁶ white blood cells/ml; elution volume 200 µl.

Protocol: Pretreatment for Whole Blood

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from fresh or frozen blood.

Starting material

This protocol is designed for processing up to 200 μ l of human whole blood. The normalization protocol (Appendix B, page 51) can be used to normalize yields from different blood samples.

Storage of blood samples

Whole blood samples treated with EDTA, ACD, or heparin* can be used, and may be either fresh or frozen. Frozen samples should be thawed at room temperature (15–25°C) with mild agitation before beginning the procedure. Yield and quality of the purified DNA depend on storage conditions of the blood. Fresher blood samples may yield better results.

- For short-term storage (up to 10 days), collect blood in tubes containing EDTA as an anticoagulant, and store the tubes at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, as low levels of DNA degradation will occur after this time.
- For long-term storage, collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store the tubes at –70°C.

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- Proteinase K is not required in this protocol.

Procedure

- 1. Thaw and equilibrate up to 48 whole blood samples at room temperature (15–25°C).**
- 2. Transfer 200 μ l of each sample into sample tubes (1.5 ml or 2 ml).**
For samples <200 μ l, bring the volume up to 200 μ l with Buffer G2.
- 3. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41, or Protocol: DNA Purification (Trace TD “Tip Dance”), page 43.**

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Protocol: Pretreatment for Dried Blood

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from dried blood. The protocol describes sample collection and the preliminary lysis of dried blood samples using proteinase K.

Starting material

Drying blood on filter paper is an effective form of storage and samples prepared in this manner are cheaper and safer to transport. A disc (3 mm diameter) punched out from filter paper stained with dried blood contains white blood cells from approximately 5 μ l whole blood; we recommend using 4 punched-out discs as starting material.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 15.

Things to do before starting

- As filter paper tends to be absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample in step 4. To provide sufficient digestion buffer for absorbent samples, Buffer G2 should be diluted with distilled water before use. Dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for $n+1$ samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 6.

Procedure

1. Collect 70 μ l of each blood sample onto a ring marked on filter paper. Allow the blood to air-dry.

Either untreated blood or blood containing an anticoagulant (EDTA, ACD, or heparin)* can be used.

2. Use the manual paper punch to cut out four 3 mm diameter discs from each dried blood sample.

3. Transfer each set of 4 discs to a 1.5 ml or 2 ml sample tube.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- 4. Add 190 μ l diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 to the sample tube until the sample volume is 190 μ l.**

Note: Prepare diluted Buffer G2 as described above in “Things to do before starting”.

- 5. Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.**
- 6. Incubate at 56°C for 15 min.**

Vortex the tube once or twice during the incubation, or place in a thermomixer.

- 7. If necessary, flick the tube to remove drops from inside the lid.**
- 8. Continue with Protocol: DNA Purification (Trace TD “Tip Dance”), page 43.**

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41.

Protocol: Pretreatment for Saliva

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from saliva samples. The protocol describes the preliminary lysis of saliva samples using proteinase K.

Starting material

The amount of saliva should not exceed 50 μ l. For larger volumes, if the sample is very dilute, see Protocol: DNA Purification (Large Volume), page 45. The normalization protocol (Appendix B, page 51) can be used to normalize yields from different saliva samples.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

1. Place up to 50 μ l saliva in a 1.5 ml or 2 ml sample tube.
2. Add 140–190 μ l Buffer G2 to the sample to bring the total volume up to 190 μ l.
3. Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.
4. Incubate at 56°C for 15 min.
Vortex the tube once or twice during the incubation, or place in a thermomixer.
5. If necessary, flick the tube to remove drops from inside the lid.
6. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41, or Protocol: DNA Purification (Trace TD “Tip Dance”), page 43.

Protocol: Pretreatment for Forensic Surface and Contact Swabs

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic surface and contact swabs. The protocol describes the preliminary lysis of forensic surface and contact swabs using proteinase K.

Starting material

Swabs may be processed on the same day as collection or stored for future processing. While storage at -20°C is recommended, DNA of suitable quality for single-copy gene amplification has been documented from swabs stored at room temperature for 24 months. The amount of biological sample material (excluding the weight of the swab) should not exceed 40 mg.

Important points before starting

- This protocol has been tested using the following swab types: plastic swabs with cotton or Dacron tips. (Puritan applicators with plastic shafts and cotton or Dacron tips are available from: Hardwood Products Company, www.hwppuritan.com, item nos. 25-806 1PC and 25-806 1PD; and from Daigger, www.daigger.com, cat. nos. EF22008D and EF22008DA). Nylon cytology brushes and other swab types may also be used.
- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- Allow the swab or brush to air-dry for at least 2 h after sample collection.
- As swabs tend to be absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample in step 1. To provide sufficient digestion buffer for absorbent samples, Buffer G2 should be diluted with distilled water before use. Dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for $n+1$ samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 3.

Procedure

- 1. Carefully cut or break off the end part of the swab or brush into a 2 ml sample tube, using an appropriate tool (e.g., scissors). Add 290 μl of diluted Buffer G2 to the sample.**

Note: Prepare diluted Buffer G2 as described above in “Things to do before starting”.

2. Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.

If processing brush samples, centrifuge the tube briefly (at 10,000 x g for 30 s) to force the brush to the bottom of the tube.

3. Incubate at 56°C for 15 min.

Vortex the tube 1–2 times during the incubation, or place in a thermomixer.

4. If necessary, flick the tube to remove drops from inside the lid.

5. Continue with Protocol: DNA Purification (Trace TD “Tip Dance”), page 43.

Using the “tip dance” protocol, there is generally no need to remove the swab or brush from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove the swab or brush from the tube. Using forceps, press the swab or brush against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41.

Protocol: Pretreatment for Nail Scrapings

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic nail-scraping samples. The protocol describes the preliminary lysis of nail-scraping samples using proteinase K.

Starting material

The amount of biological sample material should not exceed 40 mg.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

1. **Place the nail-scraping sample in a 1.5 ml or 2 ml sample tube.**
2. **Add 190 μ l Buffer G2 to the sample.**
3. **Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.**
4. **Incubate at 56°C for 15 min.**

Vortex the tube once or twice during the incubation, or place in a thermomixer.

5. **If necessary, flick the tube to remove drops from inside the lid.**
6. **Continue with Protocol: DNA Purification (Trace TD “Tip Dance”), page 43.**

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41.

Protocol: Pretreatment for Chewing Gum

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic chewing-gum samples. The protocol describes the preliminary lysis of chewing-gum samples using proteinase K.

Starting material

The amount of biological sample material (excluding the weight of the chewing gum itself) should not exceed 40 mg.

Important point before starting

- Before beginning the procedure, read "Important Notes", page 15.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

- 1. Place the chewing-gum sample in a 1.5 ml or 2 ml sample tube.**
- 2. Add 190 μ l Buffer G2 to the sample.**
- 3. Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.**
- 4. Incubate at 56°C for 15 min.**
Vortex the tube once or twice during the incubation, or place in a thermomixer.
- 5. If necessary, flick the tube to remove drops from inside the lid.**
- 6. Remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41.**

Protocol: Pretreatment for Cigarette Butts

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic cigarette-butt samples. The protocol describes the preliminary lysis of saliva and epithelial cells on paper from cigarette butts using proteinase K.

Starting material

The amount of biological sample material (excluding the weight of the paper from the cigarette butt itself) should not exceed 40 mg.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- As cigarette butts tend to be absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample in step 2. To provide sufficient digestion buffer for absorbent samples, Buffer G2 should be diluted with distilled water before use. Dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for $n+1$ samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

- 1. Place the cigarette-butt sample in a 1.5 ml or 2 ml sample tube.**
- 2. Add 190 μ l diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 to the sample tube until the sample volume is 190 μ l.**

Note: Prepare diluted Buffer G2 as described above in “Things to do before starting”.

- 3. Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.**
- 4. Incubate at 56°C for 15 min.**

Vortex the tube once or twice during the incubation, or place in a thermomixer.

- 5. If necessary, flick the tube to remove drops from inside the lid.**

6. Continue with Protocol: DNA Purification (Trace TD “Tip Dance”), page 43.

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41.

Protocol: Pretreatment for Postage Stamps

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from postage stamps. The protocol describes the preliminary lysis of postage-stamp samples using proteinase K.

Starting material

The amount of biological sample material (excluding the weight of the postage stamp itself) should not exceed 40 mg.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- As postage stamps tend to be absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample in step 2. To provide sufficient digestion buffer for absorbent samples, Buffer G2 should be diluted with distilled water before use. Dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for $n+1$ samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

- 1. Place the postage stamp in a 1.5 ml or 2 ml sample tube.**
- 2. Add 190 μ l diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 to the sample tube until the sample volume is 190 μ l.**

Note: Prepare diluted Buffer G2 as described above in “Things to do before starting”.

- 3. Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.**
- 4. Incubate at 56°C for 15 min.**

Vortex the tube once or twice during the incubation, or place in a thermomixer.

- 5. If necessary, flick the tube to remove drops from inside the lid.**

6. Continue with Protocol: DNA Purification (Trace TD “Tip Dance”), page 43.

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41.

Protocol: Pretreatment for Stains on Fabric

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from stains on fabric (e.g., blood- or saliva-stained fabrics or leather). The protocol describes the preliminary lysis of stains on fabric using proteinase K. Some samples may require larger volumes for lysis; see Protocol: DNA Purification (Large Volume), page 45.

Starting material

The amount of biological sample material (excluding the weight of the fabric itself) should not exceed 40 mg.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- As fabrics tend to be very absorbent, it is generally necessary to add a greater volume of digestion buffer to the sample in step 2. To provide sufficient digestion buffer for absorbent samples, Buffer G2 should be diluted with distilled water before use. Dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for $n+1$ samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

- 1. Place the fabric sample in a 2 ml sample tube.**
- 2. Add 190 μ l diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 to the sample tube until the sample volume is 190 μ l.**

Note: Prepare diluted Buffer G2 as described above in “Things to do before starting”.

- 3. Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.**
- 4. Incubate at 56°C for 15 min.**
Vortex the tube once or twice during the incubation, or place in a thermomixer.
- 5. If necessary, flick the tube to remove drops from inside the lid.**

6. Continue with Protocol: DNA Purification (Trace TD “Tip Dance”), page 43.

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41.

Protocol: Pretreatment for Human Tissues

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from human tissues. The protocol describes the preliminary lysis of tissues using proteinase K.

Starting material

For most tissue types, a sample size of 10 mg is recommended; however, for heart up to 20 mg and for muscle up to 40 mg may be used.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

- 1. Transfer the tissue sample into a 1.5 ml or 2 ml screw-capped tube (not supplied).**
- 2. Add 190 μ l Buffer G2.**
Ensure that tissue pieces are fully submerged in Buffer G2.
- 3. Add 10 μ l proteinase K solution and mix by tapping the tube gently.**
- 4. Incubate at 56°C until the tissue is completely lysed. Vortex 2–3 times per hour during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.**

Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 3 h. Lysis overnight is possible and does not influence the preparation.

- 5. Homogenize the sample by pipetting up and down several times. Transfer the supernatant to a new 1.5 ml or 2 ml sample tube.**
- 6. Continue with Protocol: DNA Purification (Trace TD “Tip Dance”), page 43.**

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Remove large pieces of insoluble material and centrifuge at 300 x g for 1 min. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41.

Protocol: Pretreatment for Epithelial Cells Mixed with Sperm Cells

This protocol is designed for purification of total (genomic and mitochondrial) DNA from epithelial cells mixed with sperm cells. The protocol describes the preliminary lysis of samples using proteinase K and dithiothreitol (DTT).

Starting material

Suitable starting material is epithelial cells mixed with sperm cells. The amount of biological sample material (excluding the weight of the substrate material, e.g., clothing, paper, etc.) should not exceed 40 mg.

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- As some sample types (e.g., fabrics) tend to be very absorbent, it may be necessary to add a greater volume of digestion buffer to the sample in step 2.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in steps 4 and 12.

Procedure

- 1. Place the forensic sample in a 1.5 ml or 2 ml sample tube.**
- 2. Add 190 μ l Buffer G2 to the sample.**
- 3. Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.**
- 4. Incubate at 56°C for 15 min.**

Vortex the tube once or twice during the incubation, or place in a thermomixer.

- 5. Centrifuge the tube briefly to remove drops from inside the lid.**
- 6. Remove any solid material from the tube.**

Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.

The sample volume should be approximately 200 μ l.

- 7. Centrifuge the tube at 15,000 x g for 5 min. Carefully transfer the supernatant to a new tube without disturbing the sperm cell pellet.**

DNA from epithelial cells can be purified from the tube containing the supernatant following Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41, or, if the epithelial-cell fraction is very dilute, Protocol: DNA Purification (Large Volume), page 45.

Note: The sperm cell pellet may not be visible.

- 8. Wash the sperm cell pellet by resuspending the pellet in 500 μ l Buffer G2. Centrifuge the tube at 15,000 x g for 5 min and discard the supernatant.**

- 9. Repeat step 8 two or three times.**

- 10. Add 180 μ l Buffer G2 to the pellet and resuspend the pellet.**

- 11. Add 10 μ l proteinase K and 10 μ l 1 M DTT, and mix thoroughly by vortexing for 10 s.**

- 12. Incubate at 56°C overnight at 850 rpm in a shaker-incubator or thermomixer.**

- 13. Centrifuge the tube briefly to remove drops from inside the lid. DNA from sperm cells can now be purified from this tube.**

- 14. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41.**

The two tubes in which the epithelial and sperm cells have been separated are now ready for DNA purification.

Protocol: Pretreatment for Hair

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from the root ends of plucked hair samples. The protocol describes the preliminary lysis of hair samples using proteinase K and dithiothreitol (DTT).

Starting material

The amount of biological sample material should not exceed 40 mg. We recommend using 0.5–1 cm from the root ends of plucked hair samples.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in steps 4 and 6.

Procedure

1. **Place the hair sample in a 1.5 ml or 2 ml sample tube.**
2. **Add 180 μ l Buffer G2 to the sample.**
3. **Add 10 μ l proteinase K and 10 μ l DTT solution, and mix thoroughly by vortexing for 10 s.**
4. **Incubate at 56°C for at least 6 h.**
Vortex the tube once or twice during the incubation, or place in a thermomixer.
5. **Add another 10 μ l proteinase K and 10 μ l DTT solution, and mix thoroughly by vortexing for 10 s.**
6. **Incubate at 56°C for at least 2 h until the hair samples are completely dissolved.**
7. **If necessary, flick the tube to remove drops from inside the lid.**
8. **Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41, or Protocol: DNA Purification (Trace TD “Tip Dance”), page 43.**

Protocol: Pretreatment for Bones or Teeth

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from bones or teeth. The protocol describes the preliminary grinding, decalcification using EDTA, and lysis of bone or teeth samples using proteinase K. Some samples may require larger volumes for lysis; see Protocol: DNA Purification (Large Volume), page 45.

Starting material

The amount of biological sample material should not exceed 200 mg.

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- Take time to familiarize yourself with the TissueLyser before starting this protocol. See the *TissueLyser Handbook*.

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 37°C for the decalcification in step 3.

Procedure

- 1. Remove and discard the bone or teeth surfaces. Grind the remaining bone or tooth root to a fine powder using the TissueLyser system or an equivalent bead mill.**

When using the TissueLyser, transfer the bone or tooth 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. Grind the bone or tooth sample at 30 Hz for 1 min or until the sample is pulverized (grinding times depend on type, condition, and size).

- 2. Place 150–200 mg of powdered bone into a 2 ml microcentrifuge tube.**
- 3. Add 600–700 μ l 0.5 M EDTA (pH 8.3), and incubate at 37°C for 24–48 h.**

After incubation, set the temperature to 56°C for the next incubation step.

- 4. Add 20 μ l QIAGEN Proteinase K, and incubate at 56°C for 3 h.**
- 5. Centrifuge at 6000 rpm for 4 min. Transfer 200 μ l of the supernatant to a 1.5 ml or 2 ml sample tube.**
- 6. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41, or Protocol: DNA Purification (Large Volume), page 45.**

Protocol: Pretreatment for Soil

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from soil. The protocol describes the preliminary lysis of soil samples and adsorption of inhibitors using InhibitEX tablets (contact QIAGEN Technical Services, see back cover). Some samples may require larger volumes for lysis; see Protocol: DNA Purification (Large Volume), page 45.

Starting material

The amount of biological sample material (excluding the weight of the soil itself) should not exceed 40 mg. Up to 0.5 g of soil can be used, depending on the type of soil. With flocculent soil samples, less starting material should be used.

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- Proteinase K is not required in this protocol.
- This protocol requires InhibitEX tablets (contact QIAGEN Technical Services, see back cover).

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 95°C for use in step 2.

Procedure

1. Place the soil sample in a 1.5 ml or 2 ml sample tube.
2. Add 900 μ l distilled water. Resuspend the soil by vortexing, and incubate at 95°C for 10 min.
3. Centrifuge the tube at 4000 x g for 10 min. Transfer the supernatant to another 2 ml sample tube and add 190 μ l Buffer G2. Mix by vortexing.
4. Add 1 InhibitEX tablet and incubate at room temperature (15–25°C) for 1 min.
5. Mix by vortexing and centrifuge at 10,000 x g for 2 min. Transfer 200 μ l of the supernatant to a 1.5 ml sample tube.
6. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41, or Protocol: DNA Purification (Large Volume), page 45.

Protocol: Pretreatment for Other Forensic Samples

This protocol is designed as a generic protocol for isolation of total (genomic and mitochondrial) DNA from various forensic samples. The protocol describes the preliminary lysis of samples using proteinase K.

Starting material

The amount of biological sample material (excluding the weight of the substrate material, e.g., fabric, paper, etc.) should not exceed 40 mg.

Important point before starting

- Before beginning the procedure, read “Important Notes”, page 15.

Things to do before starting

- As some sample types (e.g., bloodstained fabrics) tend to be very absorbent, it may be necessary to add a greater volume of digestion buffer to the sample in step 2. To provide sufficient digestion buffer for absorbent samples, Buffer G2 can be diluted with distilled water before use. If necessary, dilute Buffer G2 in distilled water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of distilled water) for $n+1$ samples (where n is the number of samples to be digested). Use of diluted Buffer G2 does not influence DNA yield or quality.
- Heat a thermomixer, heating block, or water bath to 56°C for the proteinase K digest in step 4.

Procedure

- 1. Place the forensic sample in a 1.5 ml or 2 ml sample tube.**
- 2. Depending on the type of sample, follow either step 2a (for non-absorbent samples) or step 2b (for absorbent samples).**
 - 2a. Non-absorbent samples:**
Add 190 μ l Buffer G2 to the sample.
 - 2b. Absorbent samples:**
Add 190 μ l diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 to the sample tube until the sample volume is 190 μ l.

Note: Prepare diluted Buffer G2 as described above in “Things to do before starting”.
- 3. Add 10 μ l proteinase K, and mix thoroughly by vortexing for 10 s.**

4. Incubate at 56°C for 15 min.

Vortex the tube once or twice during the incubation, or place in a thermomixer.

5. If necessary, flick the tube to remove drops from inside the lid.

6. Continue with Protocol: DNA Purification (Trace TD “Tip Dance”), page 43.

Using the “tip dance” protocol, there is generally no need to remove solid material from the tube.

Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 μ l. Continue with Protocol: DNA Purification (Trace Sample v3.1 or v3.0), page 41.

Protocol: DNA Purification (Trace Sample v3.1 or v3.0)

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook (pages 19–40). The protocol describes the simple procedure for setting up the BioRobot M48 and starting a run.

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- Check that Buffer MW1 has been prepared according to the instructions given on page 15.
- Before use, check that Buffer MTL does not contain a white precipitate by shaking the bottle. Check again when pipetting Buffer MTL into the reagent container. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve the precipitate.
- Buffer MW2 should not be used in this protocol. When prompted to add Buffer MW2 (in protocol “Trace Sample v3.0” only), use distilled water instead.

Things to do before starting

- Remove any solid material from the sample tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.

Procedure

1. Ensure that the BioRobot M48 is switched on.

The power switch is on the left side of the instrument.

2. Switch on the computer and monitor.

3. Launch the QIAsoft M Operating System.

Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software startup window, but this setting may have been changed.

The QIAsoft M Operating System can also be started from the QIAsoft M icon on the desktop or from the Microsoft® Windows® “Start” menu, where it is located in QIAsoft M Operating System → QIAsoft M V2.0 for BioRobot M48.

4. Select the protocol group “Forensic” from the drop-down menu by clicking the dark green arrow, then select “gDNA”.

- 5. Select the protocol “Trace Sample v3.1” or “Trace Sample v3.0”. Click the “Select” button to choose the elution tube type. Enter the number of samples, and sample and elution volumes into the software.**

QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol. Follow the steps detailed in each protocol message before continuing. Wear gloves when loading the required items on the worktable.

Note: Buffer MW2 should not be used with this protocol. When prompted to add Buffer MW2 (in protocol “Trace Sample v3.0” only), use distilled water instead.

Optional: To elute in TE buffer, add TE buffer when prompted for “RNase-free water” for elution.

- 6. Place the sample tubes on the worktable, plus reagent containers and plasticware according to the software instructions.**
- 7. Close the workstation door and start the purification procedure. All steps are fully automated, and a software message on the screen will indicate when the procedure is finished.**
- 8. Retrieve the elution tubes containing the purified DNA from the cooling block. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods.**

Generally, there is very little carryover of magnetic particles due to the small number of particles required for forensic samples. If necessary, to eliminate carried-over magnetic particles, the tube containing the eluate can first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see “Quantification of DNA”, page 49).

Protocol: DNA Purification (Trace TD “Tip Dance”)

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook (pages 19–40). This protocol describes the simple procedure for setting up the BioRobot M48 and starting a run.

In the “tip dance” protocol, the filter-tip moves back-and-forth relative to the worktable platform while pipetting. This enables processing of solid materials, such as swabs, fabrics, blood discs, or cigarette butts, directly in the sample tube. There is generally no need for prior centrifugation to remove solid materials that could clog the tip. (Alternatively, to eliminate the risk of clogging the tips, remove any solid material from the tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.)

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- Check that Buffer MW1 has been prepared according to the instructions given on page 15.
- Before use, check that Buffer MTL does not contain a white precipitate by shaking the bottle. Check again when pipetting Buffer MTL into the reagent container. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve the precipitate.
- Buffer MW2 should not be used in this protocol. When prompted to add Buffer MW2 (in protocol “Trace TD v1.0” only), use distilled water instead.

Procedure

1. Ensure that the BioRobot M48 is switched on.

The power switch is on the left side of the instrument.

2. Switch on the computer and monitor.

3. Launch the QIAsoft M Operating System.

Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software start-up window, but this setting may have been changed.

The QIAsoft M Operating System can also be started from the QIAsoft M icon on the desktop or from the Microsoft Windows “Start” menu, where it is located in QIAsoft M Operating System → QIAsoft M V2.0 for BioRobot M48.

4. Select the protocol group “Forensic” from the drop-down menu by clicking the dark green arrow, then select “gDNA”.

- 5. Select the protocol “Trace TD v1.1” or “Trace TD v1.0”. Click the “Select” button to choose the elution tube type. Enter the number of samples, and sample and elution volumes into the software.**

QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol. Follow the steps detailed in each protocol message before continuing. Wear gloves when loading the required items on the worktable.

Note: Buffer MW2 should not be used with this protocol. When prompted to add Buffer MW2 (in protocol “Trace TD v1.0” only), use distilled water instead.

Optional: To elute in TE buffer, add TE buffer when prompted for “RNase-free water” for elution.

- 6. Place the sample tubes on the worktable, plus reagent containers and plasticware according to the software instructions.**
- 7. Close the workstation door and start the purification procedure. All steps are fully automated, and a software message on the screen will indicate when the procedure is finished.**
- 8. Retrieve the elution tubes containing the purified DNA from the cooling block. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods.**

Generally, there is very little carryover of magnetic particles due to the small number of particles required for forensic samples. If necessary, to eliminate carried-over magnetic particles, the tube containing the eluate can first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see “Quantification of DNA”, page 49).

Protocol: DNA Purification (Large Volume)

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook (pages 19–40). This protocol describes the simple procedure for setting up the BioRobot M48 and starting a run.

Starting material

Using this protocol, up to 500 μl of pretreated sample can be processed. This not only allows efficient DNA purification from dilute samples with low concentrations of DNA, such as diffuse stains, but also enables purification from samples that require larger volumes for thorough lysis. For these samples, increase the amount of Buffer G2 as required. The amount of proteinase K generally does not need to be increased.

The ability to process larger sample volumes — with the same elution volume as the standard trace protocol — enables higher yields of more concentrated DNA for greater sensitivity in downstream applications.

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- This protocol requires extra Buffer MTL (contact QIAGEN Technical Services, see back cover).
- Check that Buffer MW1 has been prepared according to the instructions given on page 15.
- Before use, check that Buffer MTL does not contain a white precipitate by shaking the bottle. Check again when pipetting Buffer MTL into the reagent container. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve the precipitate.
- Buffer MW2 should not be used in this protocol. When prompted to add Buffer MW2 (in protocol “Large Volume v1.0” only), use distilled water instead.

Things to do before starting

- Remove any solid material from the sample tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.

Procedure

1. Ensure that the BioRobot M48 is switched on.

The power switch is on the left side of the instrument.

2. Switch on the computer and monitor.

3. Launch the QIAsoft M Operating System.

Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software start-up window, but this setting may have been changed.

The QIAsoft M Operating System can also be started from the QIAsoft M icon on the desktop or from the Microsoft Windows "Start" menu, where it is located in QIAsoft M Operating System → QIAsoft M V2.0 for BioRobot M48.

4. Select the protocol group "Forensic" from the drop-down menu by clicking the dark green arrow, then select "gDNA".

5. Select the protocol "Large Volume v1.1" or "Large Volume v1.0". Click the "Select" button to choose the elution tube type. Enter the number of samples, and sample and elution volumes into the software.

QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol. Follow the steps detailed in each protocol message before continuing. Wear gloves when loading the required items on the worktable.

Note: Buffer MW2 should not be used with this protocol. When prompted to add Buffer MW2 (in protocol "Large Volume v1.0" only), use distilled water instead.

Optional: To elute in TE buffer, add TE buffer when prompted for "RNase-free water" for elution.

6. Place the sample tubes (2 ml) on the worktable, plus reagent containers and plasticware according to the software instructions.

Note: Do not use 1.5 ml sample tubes with the large-volume protocol. Due to the larger sample volumes, samples in 1.5 ml tubes may overflow during pipetting.

7. Close the workstation door and start the purification procedure. All steps are fully automated, and a software message on the screen will indicate when the procedure is finished.

8. Retrieve the elution tubes containing the purified DNA from the cooling block. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods.

Generally, there is very little carryover of magnetic particles due to the small number of particles required for forensic samples. If necessary, to eliminate carried-over magnetic particles, the tube containing the eluate can first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see "Quantification of DNA", page 49).

Troubleshooting Guide

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

Comments and suggestions

General handling

QIAsoft M software error dialog box	If the QIAsoft M software displays an error dialog box during a protocol run, refer to the Troubleshooting Guide in the <i>BioRobot M48 User Manual</i> .
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Low DNA yield

- | | |
|---|---|
| a) MagAttract Suspension B not completely resuspended | Before starting the procedure, ensure that the MagAttract Suspension B is fully resuspended. Vortex for at least 3 min before first use, and for 1 min before subsequent uses. |
| b) Buffer MW1 did not contain ethanol | Ensure that the correct volume of ethanol was added to Buffer MW1. Do not use denatured ethanol, which contains other substances such as methanol or methylethylketone. Repeat the purification procedure with new samples. |
| c) Reagents loaded onto worktable in wrong order | Ensure that all reagents were loaded onto the worktable in the correct order. Repeat the purification procedure with new samples. |
| d) Purified DNA stored in water | Elute in TE buffer instead of water. Elution in TE buffer gives comparable performance and provides increased stability for long-term storage of small amounts of purified DNA. |

DNA does not perform well in downstream applications

- | | |
|---|---|
| a) Insufficient DNA used in downstream applications | If possible, repeat the downstream application using more eluate. |
|---|---|

Comments and suggestions

- b) Excess DNA used in downstream application
Excess DNA can inhibit some enzymatic reactions. Dilute the eluate or use less in the downstream application. Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see "Quantification of DNA", page 49). Use the normalization protocol (page 51) to normalize yields between samples.
- c) Buffer MW2 used instead of distilled water
Buffer MW2 should not be used in MagAttract DNA Mini M48 forensic protocols. When prompted to add Buffer MW2 (in protocols on the App. Package, M48, Forensics v2.0 only), use distilled water instead. Distilled water provides more efficient removal of PCR inhibitors during the wash steps.

A_{260}/A_{280} ratio for purified DNA is low

- a) Buffer MW1 did not contain ethanol
Ensure that the correct volume of ethanol was added to Buffer MW1. Do not use denatured ethanol, which contains other substances such as methanol or methylethylketone. Repeat the purification procedure with new samples.
- b) Absorbance reading at 320 nm not subtracted from the absorbance readings at 260 nm and 280 nm
To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see Appendix A, page 49).

Appendix A: Storage, Quantification, and Determination of Purity of DNA

Storage of DNA

Purified DNA may be stored at 2–8°C for 24 hours or at –20°C for longer storage.

Quantification of DNA

The concentration of DNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see “Spectrophotometric quantification of DNA” below). For small amounts of DNA, however, it may be difficult to determine amounts photometrically. Small amounts of DNA can be accurately quantified using an Agilent® 2100 Bioanalyzer, quantitative PCR, or fluorometric quantification using a DNA-binding dye.

Spectrophotometric quantification of DNA

Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. An absorbance of 1 unit at 260 nm corresponds to 50 μg of DNA per milliliter ($A_{260} = 1 \rightarrow 50 \mu\text{g}/\text{ml}$). Use buffer of neutral pH (e.g., 10 mM Tris·Cl, * pH 7.0) to dilute the samples and to calibrate the spectrophotometer.†

Carryover of magnetic particles in the eluate may affect the A_{260} reading, but should not affect the performance of the DNA in downstream applications. Generally, there is very little carryover of magnetic particles due to the small number of particles required for forensic samples. If necessary, to eliminate carried-over magnetic particles, the tube containing the eluate can first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see below).

To quantify DNA purified using the BioRobot M48 system:

- Apply the tube containing the DNA to a suitable magnetic separator (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912) for 1 minute. If a suitable magnetic separator is not available, centrifuge the tube containing the DNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.
- Once separation is complete, carefully withdraw 10–50 μl of purified DNA and dilute to a final volume of 100 μl in buffer of neutral pH.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

† If the samples are not diluted, use water to calibrate the spectrophotometer.

- Measure the absorbance at 320 nm and 260 nm. Subtract the absorbance reading obtained at 320 nm from the reading obtained at 260 nm to correct for the presence of magnetic particles.

Concentration of DNA sample = $50 \mu\text{g/ml} \times (A_{260} - A_{320}) \times \text{dilution factor}$

Total amount of DNA purified = concentration x volume of sample in milliliters

Purity of DNA

Purity is determined by calculating the ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm, i.e., $(A_{260} - A_{320}) / (A_{280} - A_{320})$. Pure DNA has an A_{260}/A_{280} ratio of 1.7–1.9. Use buffer of slightly alkaline pH (e.g., 10 mM Tris·Cl, pH 7.5) to dilute the samples and to calibrate the spectrophotometer.*

* If the samples are not diluted, use water to calibrate the spectrophotometer.

Appendix B: DNA Purification Protocol (Normalization)

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook (pages 19–40). This protocol describes the simple procedure for setting up the BioRobot M48 and starting a run.

Using the normalization protocol, DNA yields can be limited uniformly to 150–250 ng. This enables subsequent genetic analysis without the need to measure or adjust DNA concentration. The purified DNA is ready to use in downstream applications. The procedure has not been thoroughly tested and optimized by QIAGEN.

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 15.
- Check that Buffer MW1 has been prepared according to the instructions given on page 15.
- Before use, check that Buffer MTL does not contain a white precipitate by shaking the bottle. Check again when pipetting Buffer MTL into the reagent container. If necessary, incubate for 30 minutes at 37°C with occasional shaking to dissolve the precipitate.
- Buffer MW2 should not be used in this protocol. When prompted to add Buffer MW2 (in protocol “Normalization v1.0” only), use distilled water instead.

Things to do before starting

- Remove any solid material from the sample tube. Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.

Procedure

- 1. Ensure that the BioRobot M48 is switched on.**
The power switch is on the left side of the instrument.
- 2. Switch on the computer and monitor.**

3. Launch the QIAsoft M Operating System.

Upon startup, the computer controlling the BioRobot M48 is normally set to launch the QIAsoft M software start-up window, but this setting may have been changed.

The QIAsoft M Operating System can also be started from the QIAsoft M icon on the desktop or from the Microsoft Windows "Start" menu, where it is located in QIAsoft M Operating System → QIAsoft M V2.0 for BioRobot M48.

4. Select the protocol group "Forensic" from the drop-down menu by clicking the dark green arrow, then select "gDNA".

5. Select the protocol "Normalization v1.1" or "Normalization v1.0". Click the "Select" button to choose the elution tube type. Enter the number of samples, and sample and elution volumes into the software.

QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol. Follow the steps detailed in each protocol message before continuing. Wear gloves when loading the required items on the worktable.

Note: Buffer MW2 should not be used with this protocol. When prompted to add Buffer MW2 (in protocol "Normalization v1.0" only), use distilled water instead.

Optional: To elute in TE buffer, add TE buffer when prompted for "RNase-free water" for elution.

6. Place the sample tubes on the worktable, plus reagent containers and plasticware according to the software instructions.

7. Close the workstation door and start the purification procedure. All steps are fully automated, and a software message on the screen will indicate when the procedure is finished.

8. Retrieve the elution tubes containing the purified DNA from the cooling block. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods.

Generally, there is very little carryover of magnetic particles due to the small number of particles required for forensic samples. If necessary, to eliminate carried-over magnetic particles, the tube containing the eluate can first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see "Quantification of DNA", page 49).

Ordering Information

Product	Contents	Cat. no.
MagAttract DNA Mini M48 Kit (192)	For 192 DNA preps: MagAttract Suspension B, Buffers, Proteinase K	953336
App. Package, M48, Forensics v2.1	Software protocol package for forensics applications on the BioRobot M48 workstation	9016150
BioRobot M48	Robotic workstation for automated purification of nucleic acids using MagAttract M48 Kits, computer, installation, 1-year warranty on parts and labor*	9000708
Accessories		
Starter Pack, M48	Pack includes: sterile filter-tips (600); sample prep plates (40); large reagent containers (8); small reagent containers (8); silicon seals (8); sample tubes, 1.5 ml (250); sample tubes, 2 ml (250); elution tubes, screw cap, 1.5 ml (250); tip waste bags (2)	995999
Filter-Tips, 1000 μ l, M48 (1000)	1000 Disposable Filter-Tips for use with the BioRobot M48	995652
Reagent Containers, small, M48 (100)	Reagent containers (20 ml) with lids. To be used with the Reagent Container Rack, M48; pack of 100	995902
Reagent Containers, large, M48 (50)	Reagent containers (110 ml) with lids. To be used with the Reagent Container Rack, M48; pack of 50	995904
Reagent Container Seals, M48 (50)	Lid-sealing sheets for small and large Reagent Containers, allowing storage of unused reagents; pack of 50	995906
Sample Prep Plates, 42-well, M48 (100)	Disposable polypropylene plates for sample preparation, including nucleic acid binding and washing steps; pack of 100	995908

* Warranty PLUS 2 (cat. no. 9237714) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and parts.

Product	Contents	Cat. no.
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
Cooling Block, 48-tube, 0.2 ml, M48	Holder for accommodating 48 x 0.2 ml PCR tubes on the cooling and heating system of the BioRobot M48 worktable	9015178
Cooling Block, 48-tube, 1.4 ml, M48	Plastic holder for accommodating 48 x 1.4 ml tubes on the cooling and heating system of the BioRobot M48 worktable	9015180
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10 ml)	10 ml (>600 mAU/ml, solution)	19133
Buffer G2 (250 ml)	Lysis buffer for MagAttract DNA Mini M48 procedures	1014636
Buffer MTL (50 ml)		1023430
TissueLyser	Universal laboratory mixer-mill disruptor	85210* 85200† 85220‡
Grinding Jar Set, S. Steel (2 x 10 ml)	2 Grinding Jars (10 ml), 2 Stainless Steel Grinding Balls (20 mm)	69985

* USA and Canada.

† Japan.

‡ Rest of world.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Notes

www.qiagen.com

Australia ■ Orders 1-800-243-800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ Orders 0800-28-10-10 ■ Fax 0800-28-10-19 ■ Technical 0800-28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Brazil ■ Orders 0800-557779 ■ Fax 55-11-5079-4001 ■ Technical 0800-557779

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 86-21-3865-3865 ■ Fax 86-21-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

Italy ■ Orders 800-789-544 ■ Fax 02-334304-826 ■ Technical 800-787980

Japan ■ Telephone 03-6890-7300 ■ Fax 03-5547-0818 ■ Technical 03-6890-7300

Korea (South) ■ Orders 080-000-7146 ■ Fax 02-2626-5703 ■ Technical 080-000-7145

Luxembourg ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

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USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

