Fourth Edition

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EZ1® DNA Investigator Handbook

For automated purification of DNA from forensic and biosecurity samples using EZ1 instruments

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

EZ1 DNA Investigator Kit	**(48)**
Catalog no.	**952034**
Number of preps	**48**
Reagent Cartridge, DNA Investigator*	48
Disposable Tip Holders	50
Disposable Filter-Tips	50
Sample Tubes (2 ml)	50
Elution Tubes (1.5 ml)	50
Buffer G2	1 x 11 ml
Proteinase K	2 x 250 µl
Carrier RNA†	1 x 310 µg
Q-Card‡	1
Handbook	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 8 for safety information.

† Use of carrier RNA is optional. See "Description of protocols", page 12 and Appendix A for more information.

‡ The information encoded in the bar code on the Q-Card is needed for reagent data tracking using the EZ1 Advanced or EZI Advanced XL instrument.

Additional filter-tips and tip holders are available separately. Additional Buffer G2 and QIAGEN Proteinase K, required for some protocols, are available separately. See page 57 for ordering information.

Storage

The EZ1 DNA Investigator Kit is shipped at ambient temperature. All buffers and reagents can be stored at room temperature (15–25°C). Do not freeze the reagent cartridges. When stored properly, the reagent cartridges are stable until the expiration date on the Q-Card. Lyophilized carrier RNA is stable until the expiration date on the Q-Card when stored at room temperature.

The ready-to-use proteinase K solution is stable for up to one year after delivery when stored at room temperature.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of EZ1 DNA Investigator Kits is tested against predetermined specifications to ensure consistent product quality. Functional QC testing ensures that the EZ1 DNA Investigator Kit meets the high standards required by forensic scientists.

Product Use Limitations

The EZ1 DNA Investigator Kit is intended for molecular biology applications. This product is neither intended for the diagnosis, prevention, or treatment of a disease, nor has it been validated for such use either alone or in combination with other products.

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

Product Warranty and Satisfaction Guarantee

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

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 sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the EZ1 DNA Investigator 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 see our Technical Support Center at www.qiagen.com/goto/TechSupportCenter or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

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/Support/MSDS.aspx 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 in the reagent cartridges contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

If liquid containing potentially infectious agents is spilt on the EZ1 Advanced XL, EZ1 Advanced, or BioRobot® EZ1, 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 components of the EZ1 DNA Investigator Kit.

Reagent cartridge

Contains ethanol, guanidine hydrochloride, and guanidine thiocyanate: highly flammable, harmful, and irritant. Risk and safety phrases:* R11-20/21/22-32-36/38, S13-26-36/37/39-46

QIAGEN proteinase K

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

24-hour emergency information

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

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R11: Highly flammable; R20/21/22: Harmful by inhalation, in contact with skin, and 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; S36/37: Wear suitable protective clothing and gloves; S36/37/39: Wear suitable protective clothing, gloves, and eye/face protection; S46: If swallowed, seek medical advice immediately and show container or label.

Introduction

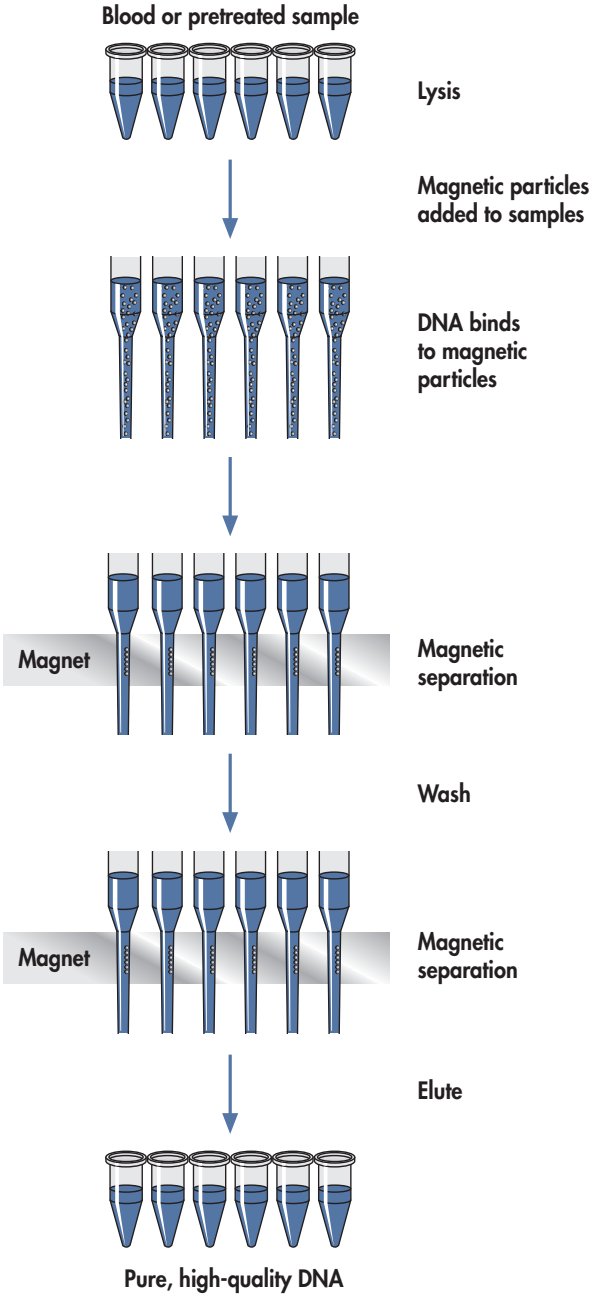
EZ1 instruments and the EZ1 DNA Investigator Kit reproducibly automate purification of genomic DNA from 1–6 samples (EZ1 Advanced and BioRobot EZ1) or 1–14 samples (EZ1 Advanced XL) encountered in forensic, human-identity, and biosecurity applications. Purification is efficient and purified DNA performs well in downstream analyses, such as quantitative PCR and STR analysis, with high signal-to-noise ratios.

Magnetic-particle technology provides high-quality DNA that is suitable for direct use in downstream applications such as STR analysis or other enzymatic reactions. EZ1 instruments perform all steps of the sample preparation procedure, and the user can choose sample input volumes of 200 µl or 500 µl, allowing purification from varying amounts of starting material. Up to 6 samples (BioRobot EZ1, EZ1 Advanced) or up to 14 samples (EZ1 Advanced XL) 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 40 µl (EZ1 Advanced XL only), 50 µl, 100 µl, or 200 µl.

EZ1 DNA Investigator Procedure



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 EZ1 instrument.
- DNA purification protocols describe setting up the EZ1 instrument and starting a fully automated run.

Pretreatment protocols

Since the type of samples that can be processed using the EZ1 DNA Investigator Kit can vary greatly, there is 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 42, 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 in water or TE buffer, with elution volumes of 40 µl (EZ1 Advanced XL only), 50 µl, 100 µl, or 200 µl. The standard **Protocol: DNA Purification (Trace Protocol)**, page 44, can be used with all sample types.

In the **Protocol: DNA Purification ("Tip Dance" Protocol)**, page 46, 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. However, when processing fluffy sample material such as cotton wool, we recommend removing solid material if you cannot process a replicate sample or the sample material is precious.

The **Protocol: DNA Purification (Large-Volume Protocol)**, page 49, 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.

The protocol for **purification of low amounts of DNA** in Appendix A, describes the optional use of carrier RNA in the purification procedure. Carrier RNA enhances binding of DNA to the silica surface of the magnetic particles, especially if the sample contains low amounts of DNA (<100 ng). Recently published data suggest that addition of carrier RNA enables more efficient isolation of low amounts of DNA from forensic samples and may, for some sample types, provide improved DNA yields. Addition of carrier RNA to sample lysates did not interfere with downstream STR analyses. 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.

All protocols

- Thermomixer, heating block, or water bath
- Vortexer
- Pipets and pipet tips (to prevent cross-contamination, we strongly recommend the use of pipet tips with aerosol barriers)
- Distilled water

For BioRobot EZ1 users

- BioRobot EZ1 instrument (cat. no. 9000705) and disposables
- EZ1 DNA Investigator Card (cat. no. 9016387)

For EZ1 Advanced users

- EZ1 Advanced instrument (cat. no. 9001410)
- EZ1 Advanced DNA Investigator Card (cat. no. 9018302)

For EZ1 Advanced XL users

- EZ1 Advanced XL instrument (cat. no. 9001492)
- EZ1 Advanced XL DNA Investigator Card (cat. no. 9018699)

For EZ1 Advanced and EZ1 Advanced XL users

For documentation purposes, one of the following is required:

- EZ1 Advanced Communicator Software (supplied with the EZ1 Advanced and EZ1 Advanced XL instruments), PC (can be connected with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments), and monitor (cat. no. for PC and monitor 9016643)
- EZ1 Advanced Communicator Software (supplied with the EZ1 Advanced and EZ1 Advanced XL instruments) and your own PC and monitor (connection with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments not recommended)
- Printer (cat. no. 9018464) and accessory package for printer (cat. no. 9018465)

For purification of DNA from dried blood

- Filter paper (e.g., QIAcard® FTA® Spots, see "Ordering Information", page 57)
- Manual paper punch, 3 mm (e.g., Harris UNI-CORE 3.00 mm Punch Kit (4), cat. no. 159331, or equivalent punch with cutting mat)

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)

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 II, cat. no. 85300, 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, large-volume protocol

- Buffer MTL (contact QIAGEN Technical Services, see back cover)

* 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 EZ1 DNA Investigator procedures can vary greatly, depending on the amount of DNA in the sample. Specific guidance for starting amounts is given in the individual protocols. EZ1 instruments can process 200 µl pretreated samples using the trace protocol (page 44) or the "tip dance" protocol (page 46) for DNA purification. With the large-volume protocol (page 49), up to 500 µl pretreated samples can be processed.

Working with EZ1 instruments

The main features of the EZ1 instruments include:

- Purification of high-quality nucleic acids from 1–6 or 1–14 samples per run
- Small footprint to save laboratory space
- Preprogrammed EZ1 Cards containing ready-to-use protocols for nucleic acid purification
- Prefilled, sealed reagent cartridges for easy, safe, and fast setup of EZ1 instruments
- Complete automation of nucleic acid purification, from opening of reagent cartridges to elution of nucleic acids, with no manual centrifugation steps

Additional features of the EZ1 Advanced and EZ1 Advanced XL include:

- Bar code reading and sample tracking
- Kit data tracking with the Q-Card provided in the kit
- UV lamp to help eliminate sample carryover from run-to-run and to allow pathogen decontamination on the worktable surfaces

Note: UV decontamination helps to reduce possible pathogen contamination of the EZ1 Advanced and EZ1 Advanced XL worktable surfaces. The efficiency of inactivation has to be determined for each specific organism and depends, for example, on layer thickness and sample type. QIAGEN cannot guarantee complete eradication of specific pathogens.

EZ1 Cards, EZ1 Advanced Cards, and EZ1 Advanced XL Cards

Protocols for nucleic acid purification are stored on preprogrammed EZ1 Cards (integrated circuit cards). The user simply inserts an EZ1 Advanced XL Card into the EZ1 Advanced XL, an EZ1 Advanced Card into the EZ1 Advanced, or an EZ1 Card into the BioRobot EZ1, and the instrument is then ready to run a protocol (Figure 1). The availability of various protocols increases the flexibility of EZ1 instruments.

Figure 1. Ease of protocol setup using EZ1 Cards. Inserting an EZ1 Card, containing a protocol, into an EZ1 instrument. The instrument should only be switched on after an EZ1 Card is inserted. EZ1 Cards should not be exchanged while the instrument is switched on.

The EZ1 DNA Investigator Kit requires use of the EZ1 Advanced XL DNA Investigator Card with the EZ1 Advanced XL, or use of the EZ1 Advanced DNA Investigator Card with the EZ1 Advanced, or use of the EZ1 DNA Investigator Card with the BioRobot EZ1. These EZ1 Cards contain protocols for purification of DNA from forensic and human-identity samples.

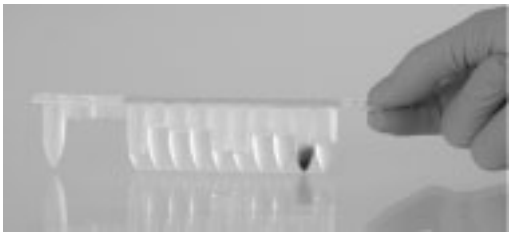
EZ1 instruments should only be switched on after an EZ1 Card is inserted. Make sure that the EZ1 Card is completely inserted (Figure 2), otherwise essential instrument data could be lost, leading to a memory error. EZ1 Cards should not be exchanged while the instrument is switched on.

Figure 2. Complete insertion of EZ1 Card. The EZ1 Card must be completely inserted before the EZ1 instrument is switched on.

Reagent cartridges

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (Figure 3). Each well of the cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Since each well contains only the required amount of reagent, generation of waste due to leftover reagent at the end of the purification procedure is avoided.

A



B

Figure 3. Ease of setup using reagent cartridges. A A sealed, prefilled reagent cartridge. Fill levels vary, depending on the type of reagent cartridge. B Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded.

Worktable

The worktable of EZ1 instruments is where the user loads samples and the components of the EZ1 DNA Investigator Kit (Figure 4).

Details on worktable setup are provided in the protocols in this handbook and are also displayed in the vacuum fluorescent display (VFD) of the EZ1 Advanced and EZ1 Advanced XL or the liquid-crystal display (LCD) of the BioRobot EZ1 control panel when the user starts worktable setup.

The display also shows protocol status during the automated purification procedure.

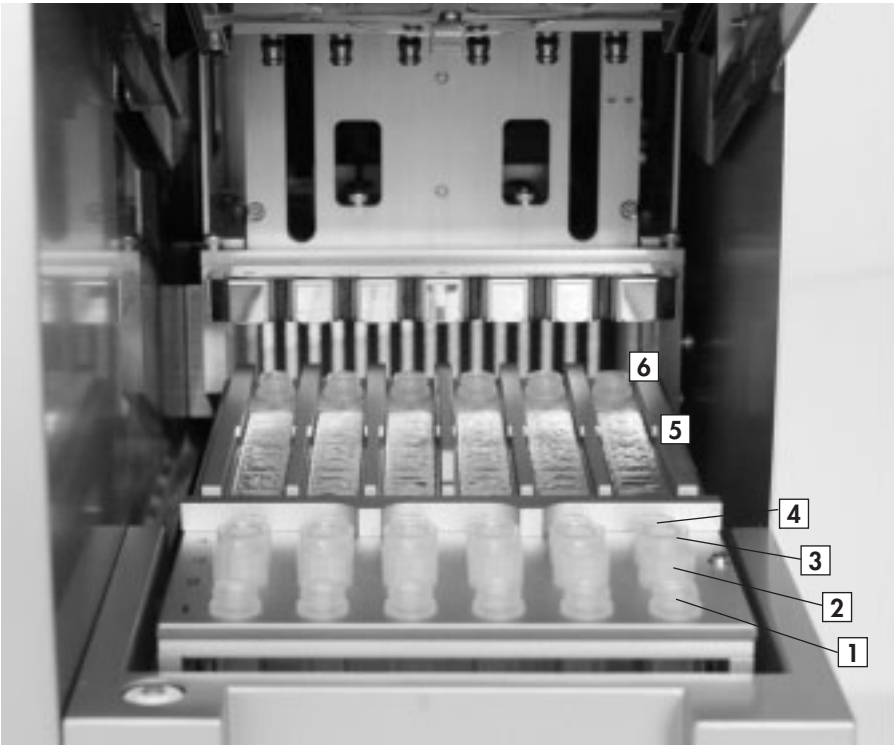


Figure 4. Typical EZ1 worktable.

1. First row: Elution tubes (1.5 ml) are loaded here.
2. Second row: Tip holders containing filter-tips are loaded here.
3. Third row: Tip holders containing filter-tips are loaded here. (In some protocols, this row is empty or loaded with 2 ml Sarstedt tubes.)
4. Fourth row: Sample tubes (2 ml) are loaded here.
5. Reagent cartridges are loaded into the cartridge rack.
6. Heating block with 2 ml tubes in the reagent cartridges for lysis.

Data tracking with the EZ1 Advanced and EZ1 Advanced XL

The EZ1 Advanced and EZ1 Advanced XL enable complete tracking of a variety of data for increased process control and reliability. The EZ1 Kit lot number and expiration date are entered at the start of the protocol using the Q-Card bar code. A user ID and the Q-Card bar code can be entered manually via the keypad or by scanning bar codes using the handheld bar code reader. Sample and assay information can also be optionally entered at the start of the protocol. At the end of the protocol run, a report file is automatically generated. The EZ1 Advanced and EZ1 Advanced XL can store up to 10 result files, and the data can be transferred to a PC or directly printed on a printer (for ordering information, see “Equipment and Reagents to Be Supplied by User” on page 13).

To receive report files on a PC, the EZ1 Advanced Communicator software needs to be installed. The software receives the report file and stores it in a folder that you define. After the PC has received the report file, you can use and process the file with a LIMS (Laboratory Information Management System) or other programs. An example of a report file is shown in Appendix B (page 55). In report files, the 6 pipetting channels of the EZ1 Advanced are named, from left to right, channels A to F or the 14 pipetting channels of the EZ1 Advanced XL are named, from left to right, channels 1–14.

When scanning a user ID or Q-Card bar code with the bar code reader, a beep confirms data input. After the information is displayed for 2 seconds, it is automatically stored, and the next display message is shown. When scanning sample ID, assay kit ID, or notes, a beep confirms data input, the information is displayed, and a message prompts you to enter the next item of information. After scanning sample ID, assay kit ID, and notes, press "ENT" once to confirm that the information entered is correct. If, for example, a wrong bar code was scanned for one of the samples, press "ESC" and then rescan all sample bar codes according to the onscreen instructions. For user ID and notes, you can enter the numbers using the keypad, or you can easily generate your own bar codes to encode these numbers.

For details about data tracking and using EZ1 Advanced Communicator software, see the *EZ1 Advanced User Manual* or the *EZ1 Advanced XL User Manual.*

Workflow of EZ1 operation

Insert EZ1 Card into the EZ1 Card slot

↓

Switch on the EZ1 instrument

↓

Follow onscreen messages for data tracking*

↓

Follow onscreen messages for worktable setup

↓

Start the protocol

↓

Collect purified nucleic acids

↓

UV decontamination*

* EZ1 Advanced and EZ1 Advanced XL only.

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. Table 1 shows typical yields for some common reference sample types.

Table 1. DNA yields from common reference sample types using EZ1 DNA Investigator procedures

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

* Whole blood with 3–7 x 10^6 white blood cells/ml; elution volume 200 µl.

Precipitate in reagent cartridge

The buffer in well 1 of the reagent cartridge (the well that is nearest to the front of the EZ1 instrument when the reagent cartridge is loaded) may form a precipitate upon storage. If necessary, redissolve by mild agitation at 37°C and then place at room temperature (15–25°C).

Equilibrating reagent cartridges

If reagent cartridges have been stored at 2–8°C, they must be equilibrated to operating temperature before use. Place the reagent cartridge into a shaker–incubator and incubate at 30–40°C with mild agitation for at least 2 hours before use. If precipitates are visible at the bottom of the wells, redissolve by incubating at 30–40°C with mild agitation for a further 2 hours. Do not use the reagent cartridges if the precipitates do not redissolve.

Lysis with proteinase K

The EZ1 DNA Investigator Kit contains proteinase K, which is the enzyme of choice for lysis buffers used in EZ1 DNA Investigator protocols. Proteinase K is a recombinant protein expressed in *Pichia pastoris* and 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 EZ1 DNA Investigator protocols.

Additional QIAGEN Proteinase K is required for purification of DNA from hair, bones, or teeth (see page 57 for ordering information).

Quantification of DNA

Depending on the sample type, the yields of DNA obtained in the purification procedure may be below 1 µg and therefore difficult to quantify using a spectrophotometer. In addition, eluates prepared with carrier RNA may contain much more carrier RNA than target nucleic acids. We recommend using quantitative amplification methods to determine yields.

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.

To eliminate carried-over magnetic particles, the tube containing the eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube.

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 µl of human whole blood.

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 6 whole blood samples at room temperature (15–25°C).**
2. **Transfer 200 µl of each sample into EZ1 sample tubes (2 ml).**
   For samples <200 µl, bring the volume up to 200 µl with Buffer G2.
3. **Continue with Protocol: DNA Purification (Trace Protocol), page 44.**

* 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 µl 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.
- Heat a thermomixer, heating block, or water bath to 95°C for use in step 7.

Procedure

1. **Collect 70 µl 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. **For each dried blood sample, use the manual paper punch to cut out four 3 mm diameter discs.**
3. **Transfer each set of 4 discs to a 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 µl 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 µl.**

   Note: Prepare diluted Buffer G2 as described in "Things to do before starting".

5. **Add 10 µl 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. **Recommended: Incubate at 95°C for 5 min.**

   Incubating the sample at 95°C may increase the yield of DNA.

8. **If necessary, flick the tube to remove drops from inside the lid.**
9. **Continue with Protocol: DNA Purification ("Tip Dance" Protocol), page 46.**

   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 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

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 µl. For larger volumes, if the sample is very dilute, see Protocol: DNA Purification (Large-Volume Protocol), page 49.

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 µl saliva in a 2 ml sample tube.**
2. **Add 140–190 µl Buffer G2 to the sample to bring the total volume up to 190 µl.**
3. **Add 10 µl 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 Protocol), page 44.**

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.

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 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.
- Heat a thermomixer, heating block, or water bath to 95°C for use in step 5.

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).**
2. **Add 290 µl of diluted Buffer G2 to the sample.**

   Note: Prepare diluted Buffer G2 as described in "Things to do before starting".
3. **Add 10 µl 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.
4. **Incubate at 56°C for 15 min.**

   Vortex the tube 1–2 times during the incubation, or place in a thermomixer.
5. **Recommended: Incubate at 95°C for 5 min.**

   Incubating the sample at 95°C may increase the yield of DNA.
6. **If necessary, flick the tube to remove drops from inside the lid.**
7. **Continue with Protocol: DNA Purification ("Tip Dance" Protocol), page 46.**

   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 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

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 2 ml sample tube.**
2. **Add 190 µl Buffer G2 to the sample.**
3. **Add 10 µl 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 ("Tip Dance" Protocol), page 46.**

   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 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

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

Use of up to 40 mg of chewing gum cut into small pieces is recommended.

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 2 ml sample tube.**
2. **Add 190 µl Buffer G2 to the sample.**
3. **Add 10 µl 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 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.**

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

Use of approximately 1 cm^2 paper from the end of the cigarette or filter is recommended.

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.
- Heat a thermomixer, heating block, or water bath to 95°C for use in step 5.

Procedure

1. **Place the cigarette-butt sample in a 2 ml sample tube.**
2. **Add 190 µl 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 µl.**

   Note: Prepare diluted Buffer G2 as described above in "Things to do before starting".
3. **Add 10 µl 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. **Recommended: Incubate at 95°C for 5 min.**

   Incubating the sample at 95°C may increase the yield of DNA.

6. **If necessary, flick the tube to remove drops from inside the lid.**
7. **Continue with Protocol: DNA Purification ("Tip Dance" Protocol), page 46.**

   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 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

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

Use of a 0.5–2.5 cm^2 piece of postage stamp is recommended.

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.
- Heat a thermomixer, heating block, or water bath to 95°C for use in step 5.

Procedure

1. **Place the piece of postage stamp in a 2 ml sample tube.**
2. **Add 190 µl 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 µl.**

   Note: Prepare diluted Buffer G2 as described above in "Things to do before starting".
3. **Add 10 µl 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. **Recommended: Incubate at 95°C for 5 min.**

   Incubating the sample at 95°C may increase the yield of DNA.

6. **If necessary, flick the tube to remove drops from inside the lid.**
7. **Continue with Protocol: DNA Purification ("Tip Dance" Protocol), page 46.**

   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 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

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 Protocol), page 49.

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.
- Heat a thermomixer, heating block, or water bath to 95°C for use in step 5.

Procedure

1. **Place the fabric sample in a 2 ml sample tube.**
2. **Add 190 µl 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 µl.**

   Note: Prepare diluted Buffer G2 as described above in "Things to do before starting".
3. **Add 10 µl 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. **Recommended: Incubate at 95°C for 5 min.**

   Incubating the sample at 95°C may increase the yield of DNA.

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

7. **Continue with Protocol: DNA Purification ("Tip Dance" Protocol), page 46.**

   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 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

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.

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 screw-capped tube (not supplied).**
2. **Add 190 µl Buffer G2.**

   Ensure that tissue pieces are fully submerged in Buffer G2.
3. **Add 10 µl 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 2 ml sample tube.**
6. **Continue with Protocol: DNA Purification ("Tip Dance" Protocol), page 46.**

   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 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

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).

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 µl Buffer G2 to the sample.**
3. **Add 10 µl 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 µl.
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 Protocol), page 44, or, if the epithelial-cell fraction is very dilute, Protocol: DNA Purification (Large-Volume Protocol), page 49.

   Note: The cell pellet may not be visible.

8. **Wash the sperm cell pellet by resuspending the pellet in 500 µl 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 µl Buffer G2 to the pellet and resuspend the pellet.**
11. **Add 10 µl proteinase K and 10 µl 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 Protocol), page 44.**

    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

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 2 ml sample tube.**
2. **Add 180 µl Buffer G2 to the sample.**
3. **Add 10 µl proteinase K and 10 µl 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 µl proteinase K and 10 µl DTT solution, and mix thoroughly by vortexing for 10 s.**
6. **Incubate at 56°C for at least 2 h or 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 ("Tip Dance" Protocol), page 46.**

   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. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

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.

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 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 at 30 Hz for 1 min or until the bone is pulverized (grinding times depend on type, condition, and size of bone).
2. **Place 150–200 mg of powdered bone into a 2 ml microcentrifuge tube.**
3. **Add 600–700 µl 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 µl QIAGEN Proteinase K, and incubate at 56°C for 3 h.**
5. **Centrifuge at 6000 rpm for 4 min. Transfer 200 µl of the supernatant to an EZ1 sample tube if proceeding with Protocol: DNA Purification (Trace Protocol) or transfer 500 µl of the supernatant to an EZ1 sample tube if proceeding with Protocol: DNA Purification (Large-Volume Protocol).**
6. **Continue with Protocol: DNA Purification (Trace Protocol), page 44, or Protocol: DNA Purification (Large-Volume Protocol), page 49.**

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).

Starting material

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 2 ml sample tube.**
2. **Add 900 µl 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 µl 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 µl of the supernatant to an EZ1 sample tube if proceeding with Protocol: DNA Purification (Trace Protocol) or transfer 500 µl of the supernatant to an EZ1 sample tube if proceeding with Protocol: DNA Purification (Large-Volume Protocol).**
6. **Continue with Protocol: DNA Purification (Trace Protocol), page 44, or Protocol: DNA Purification (Large-Volume Protocol), page 49.**

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.

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 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 µl Buffer G2 to the sample.

2b. **Absorbent samples:**

Add 190 µl 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 µl.

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

3. **Add 10 µl 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 ("Tip Dance" Protocol), page 46.**

   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 µl. Continue with Protocol: DNA Purification (Trace Protocol), page 44.

Protocol: DNA Purification (Trace Protocol)

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 22–43). The protocol describes the simple procedure for setting up the EZ1 instrument and starting a run.

Important points before starting

- If using the EZ1 DNA Investigator Kit for the first time, read "Important Notes" (page 15).
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See page 8 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of 2 choices can be made. Choose ▲ (blue) if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● (red) if using the BioRobot EZ1.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating reagent cartridges", page 20.
- 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.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. **Insert ▲ the EZ1 Advanced DNA Investigator Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Investigator Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Investigator Card completely into the EZ1 Card slot of the BioRobot EZ1.**
2. **Switch on the EZ1 instrument.**
3. **Press "START" to start protocol setup. ▲ Follow the onscreen instructions for data tracking.**
4. **Press "1" (for Trace protocol).**
5. **Choose the elution buffer and volume: press "1" to elute in water or "2" to elute in TE buffer. Then press "1", "2", or "3", (or "4", EZ1 Advanced XL only) to select the elution volume.**
6. **Press any key to proceed through the text shown on the display and start worktable setup.**

   The text summarizes the following steps which describe loading of the worktable. Wear gloves when loading the required items on the worktable.

7. **Open the instrument door.**
8. **Invert reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.**
9. **Load the reagent cartridges into the cartridge rack.**

   Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.
10. **Load opened elution tubes into the first row of the tip rack.**
11. **Load tip holders containing filter-tips into the second row of the tip rack.**
12. **Load opened sample tubes containing digested samples into the back row of the tip rack.**

    Pretreat the samples following the individual protocols in this handbook.

    Note: When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mixup.
13. **Close the instrument door.**
14. **Press "START" to start the purification procedure.**

    The automated purification procedure takes 15–20 min.
15. **When the protocol ends, the display shows "Protocol finished". ▲ Press "ENT" to generate the report file.**

    The EZ1 Advanced and the EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.
16. **Open the instrument door.**
17. **Retrieve the elution tubes containing the purified DNA. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods. Discard the sample-preparation waste.***

    If the purified DNA is to be analyzed by real-time PCR, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube in order to minimize the risk of magnetic-particle carryover.
18. **▲ Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**
19. **To run another protocol, press "ESC", prepare samples as described in the relevant protocol, and follow the procedure from step 4 onward. Otherwise, press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.**
20. **Clean the EZ1 instrument.**

    Follow the maintenance instructions in the user manual supplied with your EZ1 instrument.

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 8 for safety information.

Protocol: DNA Purification ("Tip Dance" Protocol)

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 22–43). This protocol describes the simple procedure for setting up the EZ1 instrument 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. However, when processing fluffy sample material such as cotton wool, we recommend removing solid material if you cannot process a replicate sample or the sample material is precious. (Using forceps, press the solid material against the inside of the tube to obtain maximum sample volume.)

Important points before starting

- If using the EZ1 DNA Investigator Kit for the first time, read "Important Notes" (page 15).
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See page 8 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of 2 choices can be made. Choose ▲ (blue) if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● (red) if using the BioRobot EZ1.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating reagent cartridges", page 20.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. **Insert ▲ the EZ1 Advanced DNA Investigator Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Investigator Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Investigator Card completely into the EZ1 Card slot of the BioRobot EZ1.**

2. **Switch on the EZ1 instrument.**
3. **Press "START" to start protocol setup. ▲ Follow the onscreen instructions for data tracking.**
4. **Press "2" (for Trace TD protocol).**
5. **Choose the elution buffer and volume: press "1" to elute in water or "2" to elute in TE. Then press "1", "2", or "3", (or "4", EZ1 Advanced XL only) to select the elution volume.**
6. **Press any key to proceed through the text shown on the display and start worktable setup.**

   The text summarizes the following steps which describe loading of the worktable. Wear gloves when loading the required items on the worktable.
7. **Open the instrument door.**
8. **Invert reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.**
9. **Load the reagent cartridges into the cartridge rack.**

   Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.
10. **Load opened elution tubes into the first row of the tip rack.**
11. **Load tip holders containing filter-tips into the second row of the tip rack.**
12. **Load opened sample tubes containing digested samples into the back row of the tip rack.**

    Pretreat the samples following the individual protocols in this handbook.

    Note: When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mixup.
13. **Close the instrument door.**
14. **Press "START" to start the purification procedure.**

    The automated purification procedure takes 15–20 min.
15. **When the protocol ends, the display shows "Protocol finished". ▲ Press "ENT" to generate the report file.**

    The EZ1 Advanced and the EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.
16. **Open the instrument door.**

17. **Retrieve the elution tubes containing the purified DNA. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods. Discard the sample-preparation waste.***

    If the purified DNA is to be analyzed by real-time PCR, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube in order to minimize the risk of magnetic-particle carryover.

18. **▲ Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**

19. **To run another protocol, press "ESC", prepare samples as described in the relevant protocol, and follow the procedure from step 4 onward. Otherwise, press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.**

20. **Clean the EZ1 instrument.**

    Follow the maintenance instructions in the user manual supplied with your EZ1 instrument.

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 8 for safety information.

Protocol: DNA Purification (Large-Volume Protocol)

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 22–43). This protocol describes the simple procedure for setting up the EZ1 instrument 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

- If using the EZ1 DNA Investigator Kit for the first time, read "Important Notes" (page 15).
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See page 8 for safety information.
- Perform all steps of the protocol at room temperature (15–25°C). During the setup procedure, work quickly.
- This protocol requires extra Buffer MTL (contact QIAGEN Technical Services, see back cover).
- In some steps of the procedure, one of 2 choices can be made. Choose ▲ (blue) if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● (red) if using the BioRobot EZ1.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating reagent cartridges", page 20.
- 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.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. **Insert ▲ the EZ1 Advanced DNA Investigator Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Investigator Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Investigator Card completely into the EZ1 Card slot of the BioRobot EZ1.**
2. **Switch on the EZ1 instrument.**
3. **Press "START" to start protocol setup. ▲ Follow the onscreen instructions for data tracking.**
4. **Press "3" (for Large-Volume protocol).**
5. **Choose the elution buffer and volume: press "1" to elute in water or "2" to elute in TE buffer. Then press "1", "2", or "3", (or "4", EZ1 Advanced XL only) to select the elution volume.**
6. **Press any key to proceed through the text shown on the display and start worktable setup.**

   The text summarizes the following steps which describe loading of the worktable. Wear gloves when loading the required items on the worktable.
7. **Open the instrument door.**
8. **Invert reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.**
9. **Load the reagent cartridges into the cartridge rack.**

   Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.
10. **Load opened elution tubes into the first row of the tip rack.**
11. **Load tip holders containing filter-tips into the second row of the tip rack.**
12. **Add 400 µl Buffer MTL to each sample tube containing digested samples. Load opened sample tubes containing Buffer MTL and digested samples into the back row of the tip rack.**

    Pretreat the samples following the individual protocols in this handbook.

    Note: When using the data tracking option, ensure that the sample ID follows the same order as the samples on the worktable to avoid data mixup.
13. **Close the instrument door.**
14. **Press "START" to start the purification procedure.**

    The automated purification procedure takes 15–20 min.

15. **When the protocol ends, the display shows "Protocol finished". ▲ Press "ENT" to generate the report file.**

    The EZ1 Advanced and the EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.

16. **Open the instrument door.**

17. **Retrieve the elution tubes containing the purified DNA. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods. Discard the sample-preparation waste.***

    If the purified DNA is to be analyzed by real-time PCR, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube in order to minimize the risk of magnetic-particle carryover.

18. **▲ Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**

19. **To run another protocol, press "ESC", prepare samples as described in the relevant protocol, and follow the procedure from step 4 onward. Otherwise, press "STOP" twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.**

20. **Clean the EZ1 instrument.**

    Follow the maintenance instructions in the user manual supplied with your EZ1 instrument.

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 8 for safety information.

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
General handling		
a)	Error message in instrument display	Refer to the user manual supplied with your EZ1 instrument.
b)	Report file not printed	Check whether the printer is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port. Check whether the serial port is set for use with a printer.
c)	Report file not sent to the PC	Check whether the PC is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port. Check whether the serial port is set for use with a PC.
d)	Wrong Q-Card ID entered	If the wrong ID was entered instead of the Q-Card ID, the EZ1 Advanced/EZ1 Advanced XL will not accept the ID and will prompt for the Q-Card ID until the correct ID is entered. Press "STOP" twice to go to the main menu.
Low DNA yield		
a)	Magnetic particles not completely resuspended	Ensure that you invert the reagent cartridges several times to resuspend the magnetic particles.
b)	Insufficient reagent aspirated	After inverting the reagent cartridges to resuspend the magnetic particles, ensure that you tap the cartridges to deposit the reagents at the bottom of the wells.

		Comments and suggestions
c)	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.
d)	Varying pipetting volumes	To ensure pipetting accuracy, it is important that buffer volumes in the reagent cartridges are correct and that the filter tips fit optimally to the tip adapter. Ensure that samples are thoroughly mixed and that reagent cartridges have not passed their expiry date. Perform regular maintenance as described in the instrument user manual. Check the fit of the filter tips regularly as described in the user manual.
DNA does not perform well in downstream applications		
a)	Insufficient DNA used in downstream applications	If possible, repeat the downstream application using more eluate.
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 measurement of the absorbance using an appropriate method.

Appendix A: Purification of Low Amounts of DNA

This protocol is designed for purification of isolation of total (genomic and mitochondrial) DNA from forensic samples that contain <100 ng DNA. The protocol describes the addition of carrier RNA to sample lysates. For full details, refer to Kishore, R., Hardy, W.R., Anderson, V.J., Sanchez, N.A., and Buoncristiani, M.P.H. (2006) Optimization of DNA extraction from low-yield and degraded samples using the BioRobot EZ1 and BioRobot M48. J. Forensic Sci. Vol. **51**, No. 5, 1055.

The procedure has not been thoroughly tested and optimized by QIAGEN.

Important point before starting

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

Things to do before starting

- Add 310 µl nuclease-free water or TE buffer to the tube containing carrier RNA (310 µg) to obtain a solution of 1 µg/µl.
- Dissolve the carrier RNA thoroughly, divide it into single-use aliquots, and store at –70°C.

Procedure

1. **Pretreat samples according to the appropriate pretreatment protocol given on pages 22–43 of this handbook.**
2. **Add 1 µl of thawed carrier RNA solution (1 µg) to each lysate. It is not necessary to incubate the carrier RNA and sample lysate.**
3. **Continue immediately with Protocol: DNA Purification (Trace Protocol), Protocol: DNA Purification ("Tip Dance" Protocol), or Protocol: DNA Purification (Large-Volume Protocol) on pages 44, 46, or 49 of this handbook.**

Appendix B: Example of an EZ1 Advanced Report File

This appendix shows a typical report file generated on the EZ1 Advanced. The values for each parameter will differ from the report file generated on your EZ1 Advanced. Please note that "User ID" is allowed a maximum of 9 characters, and that "Assay kit ID" and "Note" are allowed a maximum of 14 characters.

The EZ1 Advanced XL generates a similar report file containing instrument and protocol information relevant to the EZ1 Advanced XL and information for channels 1–14.

REPORT - FILE EZ1 Advanced:

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

Serial No. EZ1 Advanced: _ _ _ _ _ _ _ _ 0301F0172
User ID: _ 4121
Firmware version: _ _ _ _ _ _ _ _ _ _ _ _ _ _ V 1.0.0
Installation date of instr.: _ _ _ _ _ _ _ _ Jan 05, 2008
Weekly maintenance done on: _ _ _ _ _ Apr 15, 2008
Yearly maintenance done on: _ _ _ _ _ _ Mar 10, 2008
Date of last UV-run: _ _ _ _ _ _ _ _ _ _ _ Apr 20, 2008
Start of last UV-run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 16:06
End of last UV-run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 16:26
Status UV-run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ o.k.

Protocol name: _ _ _ _ _ _ _ _ _ _ _ _ DNA Investigator
_ Trace

Date of run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ Aprl 21, 2008
Start of run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 12:57
End of run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 13:31
Status run: _ o.k.
Error Code: _
Sample input Vol [ul]: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 200
Elution volume [ul]: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 100

Channel A:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 123456789
Reagen Kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801301
Reagen Lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ 1208
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 848373922
Note: _ 2000

Channel B:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 234567890
Reagen Kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801301
Reagen Lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1208
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 836266738
Note: _

Channel C:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 345678901
Reagen Kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801301
Reagen Lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1208
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 883727832
Notes: _1000

Channel D:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 456789012
Reagen Kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801301
Reagen Lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1208
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 763684837
Note: _

Channel E:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 567890123
Reagen Kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801301
Reagen Lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1208
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 4387728002
Note: _

Channel F:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 678901234
Reagen Kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801301
Reagen Lot number: _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1208
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 509389403
Note: _50

Ordering Information

Product	Contents	Cat. no.
EZ1 DNA Investigator Kit (48)	For 48 preps: Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers and Reagents; includes Certificate of Analysis	952034
EZ1 Advanced XL	Robotic instrument for automated purification of nucleic acids from up to 14 samples using EZ1 Kits, 1-year warranty on parts and labor*	9001492
EZ1 Advanced	Robotic instrument for automated purification of nucleic acids using EZ1 Kits, 1-year warranty on parts and labor*	9001410
EZ1 Advanced XL DNA Investigator Card	Preprogrammed card for EZ1 Advanced XL DNA Investigator protocols on the EZ1 Advanced XL	9018699
EZ1 Advanced DNA Investigator Card	Preprogrammed card for EZ1 Advanced DNA Investigator protocols	9018302
EZ1 DNA Investigator Card	Preprogrammed card for BioRobot EZ1 DNA Investigator protocols	9016387
Accessories		
Filter-Tips and Holders, EZ1 (50)	50 Disposable Filter-Tips, 50 Disposable Tip Holders; additional tips and holders for use with EZ1 Kits	994900
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
Buffer G2 (260 ml)	Lysis buffer for EZ1 DNA procedures	1014636
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10 ml)	10 ml (>600 mAU/ml, solution)	19133
TissueLyser II	Universal laboratory mixer-mill disruptor	85300

* Warranty PLUS 2 (cat. no. 9237720) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and repair parts.

Ordering Information

Product	Contents	Cat. no.
Grinding Jar Set, S. Steel (2 x 10 ml)	2 Grinding Jars (10 ml), 2 Stainless Steel Grinding Balls (20 mm)	69985
PC and TFT Monitor, 17"	PC capable of connection with up to 4 EZ1 Advanced or EZ1 Advanced XL instruments; Monitor for use with PC	9016643
Printer	Printer for connection with EZ1 Advanced or EZ1 Advanced XL instrument	9018464
Printer Accessory Package	Accessories for printer connected to EZ1 Advanced or EZ1 Advanced XL instrument	9018465
QIAcard FTA One Spot (100)	For collection and storage of 100 samples: 100 QIAcard FTA One Spots	159201
QIAcard FTA Two Spots (100)	For collection and storage of 100 x 2 samples: 100 QIAcard FTA Two Spots	159203
QIAcard FTA Four Spots (100)	For collection and storage of 100 x 4 samples: 100 QIAcard FTA Four Spots	159205
QIAcard FTA Indicator Four Spots (25)	For collection and storage of 25 x 4 samples: 25 QIAcard FTA Indicator Four Spots	159214
QIAcard FTA Purification Reagent (500 ml)	For use with QIAcard FTA Spots: 500 ml QIAcard FTA Purification Reagent	159300
Related products		
EZ1 DNA Blood 200 µl Kit (48)	48 Reagent Cartridges, 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes, 50 Elution Tubes	951034
EZ1 DNA Blood 350 µl Kit (48)	48 Reagent Cartridges, 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes, 50 Elution Tubes	951054

Ordering Information

Product	Contents	Cat. no.
EZ1 DNA Tissue Kit (48)	48 Reagent Cartridges, 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes, 50 Elution Tubes, Buffer G2, Proteinase K	953034
EZ1 Virus Mini Kit v2.0 (48)	For 48 virus nucleic acid preps: Reagent Cartridges (Virus Mini v2.0), Disposable Tips, Disposable Tip-Holders, Sample Tubes, Elution Tubes, Buffers	955134

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