EZ1® DNA Investigator® Handbook

For automated purification of DNA from forensic and biosecurity samples using EZ1 instruments
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.
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<th>(48)</th>
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<tr>
<td>Catalog no.</td>
<td>952034</td>
</tr>
<tr>
<td>Number of preps</td>
<td>48</td>
</tr>
<tr>
<td>Reagent Cartridge, DNA Investigator*</td>
<td>48</td>
</tr>
<tr>
<td>Disposable Tip Holders</td>
<td>50</td>
</tr>
<tr>
<td>Disposable Filter-Tips</td>
<td>50</td>
</tr>
<tr>
<td>Sample Tubes (2 ml)</td>
<td>50</td>
</tr>
<tr>
<td>Elution Tubes (1.5 ml)</td>
<td>50</td>
</tr>
<tr>
<td>Buffer G2</td>
<td>2 x 11 ml</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>1 x 1.2 ml</td>
</tr>
<tr>
<td>Carrier RNA</td>
<td>1 x 310 μg</td>
</tr>
<tr>
<td>Q-Card‡</td>
<td>1</td>
</tr>
<tr>
<td>Handbook</td>
<td>1</td>
</tr>
</tbody>
</table>

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for “Safety 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, required for some protocols, is available separately. See page 42 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 1 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 safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

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

Buffers 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.
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) can be 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 8). 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

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 2 types of protocols (Table 1).

- 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.

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)” can be used with all sample types (page 28).

In the “Protocol: DNA Purification (Tip Dance Protocol)”, page 31, 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 if the sample material is precious.

The “Protocol: DNA Purification (Large-Volume Protocol)”, page 34, enables fully automated processing of starting volumes up to 500 μl. This allows efficient DNA purification from dilute samples with low concentrations of DNA, such as diffuse stains, as well as 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.
Table 1. Protocol information for different sample types

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Pre-treatment protocols</th>
<th>Purification protocols</th>
<th>Sample amount</th>
<th>Buffer G2</th>
<th>Proteinase K</th>
<th>DTT 1 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood/saliva</td>
<td>Page 21</td>
<td>Trace</td>
<td>Up to 50 μl</td>
<td>140–190 μl</td>
<td>10 μl</td>
<td>no</td>
</tr>
<tr>
<td>FTA® cards</td>
<td>Page 21</td>
<td>Trace or Tip Dance</td>
<td>4 x 3mm punches</td>
<td>290 μl</td>
<td>10 μl</td>
<td>no</td>
</tr>
<tr>
<td>Surface swabs</td>
<td>Page 21</td>
<td>Trace or Tip Dance</td>
<td>1 swab</td>
<td>290 μl</td>
<td>10 μl</td>
<td>no</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>Page 21</td>
<td>Trace or Tip Dance</td>
<td>Up to 40 mg</td>
<td>190 μl</td>
<td>10 μl</td>
<td>no</td>
</tr>
<tr>
<td>Cigarette butts</td>
<td>Page 21</td>
<td>Trace or Tip Dance</td>
<td>1 cm²</td>
<td>190 μl</td>
<td>10 μl</td>
<td>no</td>
</tr>
<tr>
<td>Paper/similar materials</td>
<td>Page 21</td>
<td>Trace or Tip Dance</td>
<td>0.5–2.5 cm²</td>
<td>190 μl</td>
<td>10 μl</td>
<td>no</td>
</tr>
<tr>
<td>Nail scrapings</td>
<td>Page 21</td>
<td>Trace</td>
<td>Up to 40 mg</td>
<td>190 μl</td>
<td>10 μl</td>
<td>no</td>
</tr>
<tr>
<td>Nail clippings</td>
<td>Page 21</td>
<td>Trace</td>
<td>1</td>
<td>160 μl</td>
<td>20 μl</td>
<td>20 μl</td>
</tr>
<tr>
<td>Hair</td>
<td>Page 21</td>
<td>Trace</td>
<td>0.5–1cm</td>
<td>160 μl</td>
<td>20 μl</td>
<td>20 μl</td>
</tr>
<tr>
<td>Tissues</td>
<td>Page 21</td>
<td>Trace</td>
<td>Up to 10 mg</td>
<td>190 μl</td>
<td>10 μl</td>
<td>no</td>
</tr>
<tr>
<td>Blood or saliva stains</td>
<td>Page 21</td>
<td>Trace or Tip Dance</td>
<td>0.5 cm²</td>
<td>290 μl</td>
<td>10 μl</td>
<td>no</td>
</tr>
<tr>
<td>Semen stains</td>
<td>Page 21</td>
<td>Trace or Tip Dance</td>
<td>0.5 cm²</td>
<td>270 μl</td>
<td>10 μl</td>
<td>20 μl</td>
</tr>
<tr>
<td>Large volume</td>
<td>Page 21</td>
<td>Large-Volume</td>
<td>Varies</td>
<td>475 μl</td>
<td>25 μl</td>
<td>no</td>
</tr>
<tr>
<td>Large volume semen</td>
<td>Page 21</td>
<td>Large-Volume</td>
<td>Varies</td>
<td>455 μl</td>
<td>25 μl</td>
<td>20 μl</td>
</tr>
<tr>
<td>Sexual assault samples</td>
<td>Page 23</td>
<td>Trace</td>
<td>Varies</td>
<td>up to 2.5 ml*</td>
<td>20 μl</td>
<td>40 μl</td>
</tr>
<tr>
<td>Bones or teeth</td>
<td>Page 25</td>
<td>Trace</td>
<td>150–200 mg</td>
<td>0.5 M EDTA</td>
<td>20 μl</td>
<td>no</td>
</tr>
<tr>
<td>Soil</td>
<td>Page 27</td>
<td>Trace or Tip Dance</td>
<td>Up to 0.5 g</td>
<td>100 μl</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

* Depends on number of sperm pellet washes.
Equipment and Reagents to Be Supplied by User

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

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)

For BioRobot EZ1 users

- EZ1 DNA Investigator Card (cat. no. 9016387)

For EZ1 Advanced users

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

For EZ1 Advanced XL users

- EZ1 Advanced XL DNA Investigator Card (cat. no. 9018699) or EZ1 Advanced XL DNA Investigator Flip-Cap Card (cat.no. 9022763)
- Optional: EZ1 Advanced XL Flip-Cap Tube Rack (cat. no. 9022818)

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)
For purification of DNA from epithelial cells mixed with sperm cells
- Buffer G2, cat. no. 1014636
- 1 M dithiothreitol (DTT)
- Microcentrifuge

For purification of DNA from hair
- 1 M dithiothreitol (DTT)

For purification of DNA from bones or teeth
- 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 (54 ml) (cat. no.19112)
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 and Table 1. EZ1 instruments can process 200 μl pretreated samples using the “Trace Protocol”, (page 28) or the “Tip Dance Protocol”, (page 31) for DNA purification. With the “Large-Volume Protocol”, (page 34), up to 500 μl pretreated samples can be processed.

Purification of low amounts of DNA
For purification of DNA from very small amounts of sample, such as low volumes of blood (<10 μl) or forensic casework samples, we recommend adding carrier RNA. For samples containing larger amounts of DNA, addition of carrier RNA is optional. Add 310 μl TE buffer or water to the tube containing 310 μg lyophilized carrier RNA to obtain a solution of 1 μg/μl. Dissolve the carrier RNA thoroughly, divide into conveniently sized aliquots, and store at −20°C. Do not freeze-thaw the aliquot of carrier RNA more than 3 times. Carrier RNA should be added to the sample after the lysis is completed to avoid degradation. When using the “Large-Volume Protocol”, carrier RNA can be added to buffer MTL prior to setting up the instrument run. Use the mixture of MTL and carrier RNA within the same day.

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 (see Figure 1). The availability of various protocols increases the flexibility of EZ1 instruments. Note that the Flip-Cap Tube Rack can only be used with the EZ1 DNA Investigator Flip-Cap card on an EZ1 ADV XL.

![EZ1 Card](image1.png)

**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 (see 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](image)

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 (see 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.
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 (see Figure 4). For the EZ1 Advanced XL 2 sample racks are available: The standard rack for use with the screw cap sample and elution tubes provided with the EZ1 DNA Investigator Kit, and a Flip-Cap Tube Rack that can be used with both, screw cap and Flip-Cap Tubes. Please note that the Flip-Cap Tube Rack can only be used with the EZ1 DNA Investigator Flip-Cap Card. Details on worktable setup are provided in the protocols in this handbook and 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 11).

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 LIMS (Laboratory Information Management System) or other programs. An example of a report file is shown in “Appendix A”, (page 39). 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 re-scan all sample bar codes according to the on-screen 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 on-screen messages for data tracking*
↓
Follow on-screen 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 2 shows typical yields for some common reference sample types.

Table 2. DNA yields from common reference sample types using EZ1 DNA Investigator procedures

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample amount</th>
<th>Protocol</th>
<th>DNA yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood*</td>
<td>10–200 μl</td>
<td>Trace or Tip Dance</td>
<td>150 ng–2 μg</td>
</tr>
<tr>
<td>Dried blood</td>
<td>4 x 3 mm disc</td>
<td>Tip Dance</td>
<td>0.2–0.5 μg</td>
</tr>
<tr>
<td>Buccal cells</td>
<td>1 swab</td>
<td>Tip Dance</td>
<td>100 ng–2 μg</td>
</tr>
</tbody>
</table>

* Whole blood with 3–7 x 10⁶ 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.

Quantification of DNA

Degraded, inhibited, or mixed DNA samples are common in forensic casework and other human identity testing applications. Such samples can create challenges in STR analysis. Prior quantification of the purified DNA using real-time PCR is recommended and reduces the need to repeat downstream analyses. This greatly reduces costs and time and improves the statistical relevance of results. Investigator Quantiplex™ Kits, use quantitative real-time PCR to quantify human DNA in a sample. These kits also detect if a sample contains sufficient DNA to enable DNA fingerprinting analysis and if the sample contains inhibitors that may interfere with downstream applications. They are designed for use in a whole range of human identity and forensics applications, and are available in 2 versions: the Investigator Quantiplex Kit, for quantification of total human DNA; or the Investigator Quantiplex HYres Kit, for quantification of total human and human male DNA.
Protocol: Pretreatment for Various Casework and Reference Samples

This protocol is designed for isolation of total DNA (genomic and mitochondrial) from various types of casework and reference samples. The protocol describes the preliminary lysis using Proteinase K.

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 13.
- We recommend using the Investigator Lyse&Spin Basket Kit (cat. no. 19597) when solid sample materials have to be removed from the lysate. If using this kit, please follow the Pretreatment Protocol described in the corresponding handbook and the “Large-Volume Protocol” for DNA purification. The Lyse&Spin Basket Kit collection tubes can be used as sample tubes for the EZ1 Advanced XL, using the Flip-Cap Tube Rack (cat. no. 9022818) and Flip-Cap Card (cat. no. 9022763).

Things to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the Proteinase K digest in step 3.

Procedure

1. Place the sample in a 2 ml sample tube.
2. Set up the Proteinase K digest according to information given in Table 1, page 10. Mix sample thoroughly by vortexing for 10 s.
3. Incubate at 56°C for 15 min to overnight in a thermomixer shaking at 900 rpm.
   
   15 min may be sufficient to recover adequate DNA for STR typing from samples containing abundant DNA. More than 1 h is recommended where a low amount of DNA is expected.

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

   Optional: Add 1 μg carrier RNA (see “Important Notes”, page 13)

5. Continue with “Protocol: DNA Purification”, using one of the following options:


   For samples that do not contain solid materials. The lysate volume should be approximately 200 μl.

When using the “Tip Dance Protocol”, there is generally no need to remove solid material from the tube. 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. Note that the “Tip Dance Protocol” will not recover the lysate absorbed by the sample substrate (e.g. swab, piece of fabric), therefore, a slightly reduced sensitivity has to be expected compared to methods that fully recover the lysate. We recommend using the Investigator Lyse&Spin Basket Kit for maximum sensitivity.

c) “Large-Volume Protocol”, page 34.

The “Large-Volume Protocol” purifies DNA from 500 μl lysate.
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 13.
- 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 for the proteinase K digest to 56°C in steps 4 and 70°C in step 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 1–2 h. Do not exceed 2 h.
   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.
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 28, or, if the epithelial cell fraction is very dilute, “Protocol: DNA Purification (Large-Volume Protocol)”, page 34. 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 160 μl Buffer G2 to the pellet and resuspend the pellet.
11. Add 10 μl Proteinase K and 40 μl 1 M DTT, and mix thoroughly by vortexing for 10 s.
12. Incubate at 70°C for 10 min at 850 rpm in a shaker–incubator or thermomixer.
   For maximum recovery, place samples in an Ultrasonicator for 10 min. Alternatively, vortex vigorously for 10 s.
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 28. The two tubes in which the epithelial and sperm cells have been separated are now ready for DNA purification.
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 13.

■ 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 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)”.
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 13.
- 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)”.
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 21–27). 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 13).
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See page 6 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 ▲ if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● 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.
- 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 on-screen instructions for data tracking.
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 on-screen 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 6 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 21–27). 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.

Important points before starting

- If using the EZ1 DNA Investigator Kit for the first time, read “Important Notes”, (page 13).
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See page 6 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 ▲ if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● 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 on-screen 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 on-screen 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 6 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 21–27). 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. 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 13).
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See page 6 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 (cat. no. 19112)
- In some steps of the procedure, one of 2 choices can be made. Choose ▲ if using the EZ1 Advanced or the EZ1 Advanced XL; choose ● 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.
- 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 on-screen instructions for data tracking.


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.
   **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 on-screen 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 6 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 “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 printer is connected to the EZ1 Advanced or EZ1 Advanced XL via “PC/Printer” serial port.
Check whether the serial port is set for use with a printer.

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.
## Comments and suggestions

| 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. |
| 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 applications | 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: 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
.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: Apr 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
Reagent Kit number: 9801301
Reagent Lot number: 23456789
Reagent Expiry date: 1208
Assay kit ID: 848373922
Note: 2000

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

Channel C:
Sample ID: 345678901
Reagent Kit number: 9801301
Reagent Lot number: 23456789
Reagent Expiry date: 1208
Assay kit ID: 883727832
Notes: 1000

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

Channel E:
Sample ID: 567890123
Reagent Kit number: 9801301
Reagent Lot number: 23456789
Reagent Expiry date: ________________ 1208
Assay kit ID: ______________________ 4387728002
Note: ______________________________________________________

Channel F:
Sample ID: ______________________ 678901234
Reagent Kit number: ________________ 9801301
Reagent Lot number: ________________ 23456789
Reagent Expiry date: ________________ 1208
Assay kit ID: ______________________ 509389403
Note: _______________________________ 50
## Ordering Information

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<thead>
<tr>
<th>Product</th>
<th>Contents</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EZ1 DNA Investigator Kit (48)</td>
<td>For 48 preps: Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers, and Reagents; includes Certificate of Analysis</td>
<td>952034</td>
</tr>
<tr>
<td>EZ1 Advanced XL</td>
<td>Robotic instrument for automated purification of nucleic acids from up to 14 samples using EZ1 Kits, 1-year warranty on parts and labor*</td>
<td>9001492</td>
</tr>
<tr>
<td>EZ1 Advanced XL Flip-Cap Tube Rack</td>
<td>Tube rack for the use of Flip-Cap tubes on EZ1 Advanced XL</td>
<td>9022818</td>
</tr>
<tr>
<td>EZ1 Advanced XL DNA Investigator Flip-Cap Card</td>
<td>Preprogrammed card for purification of DNA using the EZ1 Advanced XL with an EZ1 Advanced XL Flip-Cap Tube Rack</td>
<td>9022763</td>
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<tr>
<td>EZ1 Advanced XL DNA Investigator Card</td>
<td>Preprogrammed card for EZ1 Advanced XL DNA Investigator protocols on the EZ1 Advanced XL</td>
<td>9018699</td>
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</tr>
<tr>
<td>Filter-Tips and Holders, EZ1 (50)</td>
<td>50 Disposable Filter-Tips, 50 Disposable Tip Holders; additional tips and holders for use with EZ1 Kits</td>
<td>994900</td>
</tr>
<tr>
<td>12-Tube Magnet</td>
<td>Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes</td>
<td>36912</td>
</tr>
</tbody>
</table>

* 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.
<table>
<thead>
<tr>
<th>Product</th>
<th>Contents</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer G2 (260 ml)</td>
<td>Lysis buffer for EZ1 DNA procedures</td>
<td>1014636</td>
</tr>
<tr>
<td>MTL Buffer (50 ml)</td>
<td></td>
<td>19112</td>
</tr>
<tr>
<td>QIAGEN Proteinase K (1.2 ml)</td>
<td>1.2 ml (&gt;600 mAU/ml, solution)</td>
<td>1014023</td>
</tr>
<tr>
<td>Tissuelyser II</td>
<td>Universal laboratory mixer-mill disruptor</td>
<td>85300</td>
</tr>
<tr>
<td>Grinding Jar Set, S. Steel (2 x 10 ml)</td>
<td>2 Grinding Jars (10 ml), 2 Stainless Steel Grinding Balls (20 mm)</td>
<td>69985</td>
</tr>
<tr>
<td>PC and TFT Monitor, 17”</td>
<td>PC capable of connection with up to 4 EZ1 Advanced or EZ1 Advanced XL instruments; Monitor for use with PC</td>
<td>9016643</td>
</tr>
<tr>
<td>Printer</td>
<td></td>
<td>9018464</td>
</tr>
</tbody>
</table>

**HID related products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Contents</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Investigator Quantiplex Kit (200)</td>
<td>Primer mix IC FQ, reaction mix FQ, control DNA Z1, dilution buffer</td>
<td>387016</td>
</tr>
<tr>
<td>EZ1 DNA Blood 350 μl Kit (48)</td>
<td>Primer mix IC YQ, reaction mix FQ, control DNA Z1, dilution buffer</td>
<td>387116</td>
</tr>
</tbody>
</table>

**Non-HID related products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Contents</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EZ1 DNA Blood 200 μl Kit (48)</td>
<td>48 Reagent Cartridges, 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes, 50 Elution Tubes</td>
<td>951034</td>
</tr>
<tr>
<td>EZ1 DNA Blood 350 μl Kit (48)</td>
<td>48 Reagent Cartridges, 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes, 50 Elution Tubes</td>
<td>951054</td>
</tr>
<tr>
<td>Product</td>
<td>Contents</td>
<td>Cat. no.</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>EZ1 DNA Tissue Kit (48)</td>
<td>48 Reagent Cartridges, Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes, 50 Elution Tubes, Buffer G2, Proteinase K</td>
<td>95303450</td>
</tr>
</tbody>
</table>

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