

EZ1[®] DNA Blood Handbook

EZ1 DNA Blood 200 μ l Kit

EZ1 DNA Blood 350 μ l Kit

For automated purification of DNA from
blood and buffy coat 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

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Contents

Kit Contents	4
Storage	4
Product Use Limitations	4
Product Warranty and Satisfaction Guarantee	5
Technical Assistance	5
Safety Information	6
Quality Control	6
Introduction	7
Principle and procedure	7
Equipment and Reagents to Be Supplied by User	9
Important Notes	10
Starting material	10
Precipitate in reagent cartridge	11
Quantification of DNA	11
Working with EZ1 instruments	11
Yield of purified DNA	17
Protocols	
■ Purification of DNA from Whole Blood	18
■ Purification of DNA from Buffy Coat	21
Troubleshooting Guide	25
Appendix A: Storage, Quantification, and Determination of Purity of DNA	27
Appendix B: Example of an EZ1 Advanced Report File	28
Ordering Information	30

Kit Contents

EZ1 DNA Blood Kits*	Blood 200 μl	Blood 350 μl
Catalog no.	951034	951054
Preps per kit	48	48
Reagent Cartridge, Blood 200 μ l (1023745)	48	—
Reagent Cartridge, Blood 350 μ l (1023729)	—	48
Disposable Tip Holders	50	50
Disposable Filter-Tips	50	50
Sample Tubes (2 ml)	50	50
Elution Tubes (1.5 ml)	50	50
Q-Card [†]	1	1
Handbook	1	1

* For details about the EZ1 Cards to be used with these kits, see Table 2, page 13, and visit www.qiagen.com.

[†] The information encoded in the bar code on the Q-Card is needed for reagent data tracking using the EZ1 Advanced and EZ1 Advanced XL instruments.

Storage

The EZ1 DNA Blood Kits are 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.

Product Use Limitations

The EZ1 DNA Blood 200 μ l Kit and EZ1 DNA Blood 350 μ l Kit are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

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

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 EZ1 DNA Blood Kits 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/Support 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 hydrochloride/guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. If liquid containing potentially infectious agents is spilt on the EZ1 instrument, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

The following risk and safety phrases apply to the components of the EZ1 DNA Blood Kits:

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

24-hour emergency information

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

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of EZ1 DNA Blood Kits is tested against predetermined specifications to ensure consistent product quality.

* 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. S13: Keep away from food, drink, and animal feedingstuffs; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S46: If swallowed, seek medical advice immediately and show this container or label.

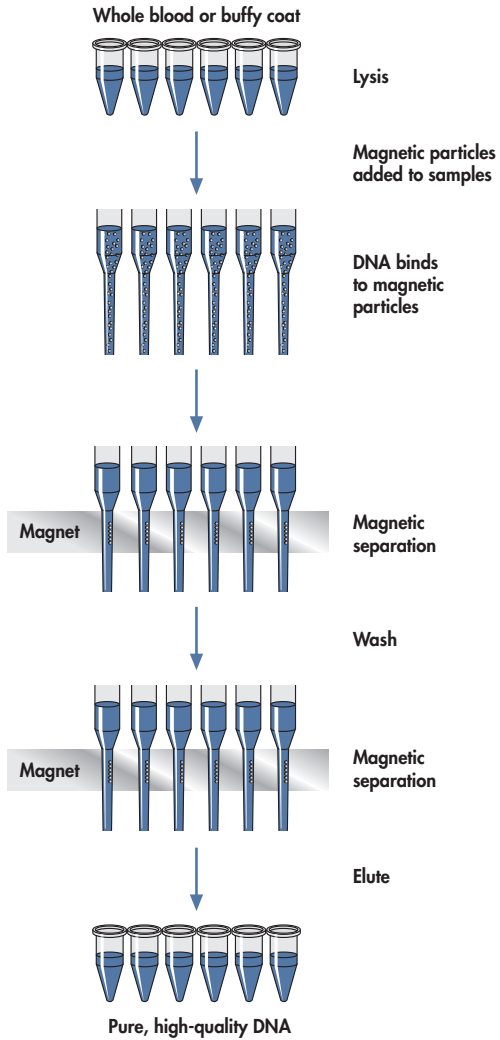
Introduction

The EZ1 DNA Blood 200 μ l Kit and EZ1 DNA Blood 350 μ l Kit are for purification of genomic DNA from whole blood samples and buffy coat. Magnetic-particle technology provides high-quality DNA that is suitable for direct use in downstream applications such as amplification or other enzymatic reactions. The EZ1 instruments perform all steps of the sample preparation procedure, and the procedure can be scaled up or down, allowing purification from varying amounts of starting material. Up to 6 samples (using BioRobot EZ1 and EZ1 Advanced instruments) or up to 14 samples (using the EZ1 Advanced XL instrument) 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 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 elution buffer.

EZ1 DNA Blood Procedure



Equipment and Reagents to Be Supplied by User

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

All protocols

- Sterile, RNase-free pipet tips
- Soft paper tissue
- Water
- 70% ethanol
- Pipets
- Thermomixer, heated orbital incubator, heating block, or water bath

For BioRobot EZ1 users

- BioRobot EZ1 (cat. no. 9000705) and disposables
- EZ1 Card (see Table 2, page 13)

For EZ1 Advanced users

- EZ1 Advanced (cat. no. 9001410 or 9001411)
- EZ1 Advanced Card (see Table 2, page 13)

For EZ1 Advanced XL users

- EZ1 Advanced XL (cat. no. 9001492)
- EZ1 Advanced XL Card (see Table 2, page 13)

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 the EZ1 Advanced XL), 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 the EZ1 Advanced XL) 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 Blood Protocols

- Optional: 80% ethanol, 2 ml screw-capped tubes (if optional 80% ethanol wash step is performed, see "Important points before starting", page 18).

For Buffy Coat Protocol

- 1.5 ml or 2 ml screw-capped tube

Important Notes

Starting material

The amounts of starting material for use in EZ1 DNA Blood procedures are shown in Table 1.

Table 1. Amounts of starting material for EZ1 DNA Blood procedures

Sample type	Amount of starting material	Elution volume
Blood	200 μ l or 350 μ l	50 μ l, 100 μ l, or 200 μ l
Buffy coat: [*]		
Buffy coat, enriched >9x [†]	50–75 μ l	200 μ l
Buffy coat, enriched \leq 9x [†]	100–150 μ l	200 μ l
Buffy coat with low leukocyte concentration [§]	200–300 μ l	200 μ l

^{*} For each buffy coat protocol, the maximum number of cells to use as starting material is 5×10^6 cells.

[†] For example, 1 ml leukocyte containing fraction harvested from 10 ml centrifuged whole blood = 10x enrichment.

[‡] This is recommended for preparation of buffy coat.

[§] For example, from certain leukemia patients or other donors where leukocyte count is low.

Storage of blood

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.

[†] 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.

Buffy coat

Buffy coat may vary considerably in leukocyte concentration depending upon the number of nucleated cells in the original whole blood sample and the efficiency of leukocyte harvesting during the buffy coat preparation. In order to avoid overloading the isolation procedure, if using highly enriched buffy coat samples (>9x enrichment), smaller volumes of starting material should be used (see Table 1, page 10 for recommended starting volumes). Efficiency of buffy coat enrichment depends on the sample preparation procedure used and on the accuracy used when extracting the buffy coat layer. Three different protocols are available for purification of genomic DNA from buffy coat on EZ1 instruments. The amounts of recommended starting material for the 3 different protocols are shown in Table 1.

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.

Quantification of DNA

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A_{260}) of the purified DNA but should not affect downstream applications. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. See "Quantification of DNA", page 27, for more information.

Working with EZ1 instruments

The main features of 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 the 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

Protocols for nucleic acid purification are stored on preprogrammed EZ1 Cards (integrated circuit cards). The user simply inserts an EZ1 Card into the BioRobot EZ1, or an EZ1 Advanced Card into the EZ1 Advanced, or an EZ1 Advanced XL Card into the EZ1 Advanced XL, 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 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.

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.

The EZ1 Kit and EZ1 Card required depend on the purification procedure to be carried out and the EZ1 instrument used (Table 2).

Table 2. Purification of DNA from various sample types

Sample type	EZ1 Card required	EZ1 Kit required
Blood (200 µl)	EZ1 Advanced XL DNA Blood Card,* EZ1 Advanced DNA Blood Card,† or EZ1 DNA Blood Card‡	EZ1 DNA Blood 200 µl Kit
Blood (350 µl)	EZ1 Advanced XL DNA Blood Card,* EZ1 Advanced DNA Blood Card,† or EZ1 DNA Blood Card‡	EZ1 DNA Blood 350 µl Kit
Buffy coat	EZ1 Advanced XL DNA Buffy Coat Card,* EZ1 Advanced DNA Buffy Coat Card,† or EZ1 DNA Buffy Coat Card‡	EZ1 DNA Blood 350 µl Kit

* EZ1 Advanced XL Cards are only for use with the EZ1 Advanced XL.

† EZ1 Advanced Cards are only for use with the EZ1 Advanced.

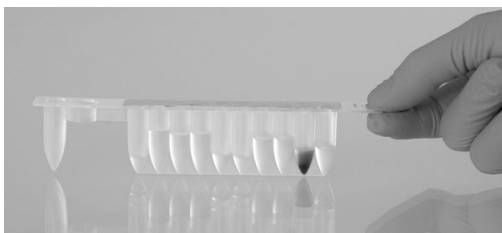
‡ EZ1 Cards are only for use with the BioRobot EZ1.

Visit www.qiagen.com/literature for the latest supplementary protocols, which describe how to use EZ1 DNA Kits and EZ1 DNA Cards for other applications.

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 Kits (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 the 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.

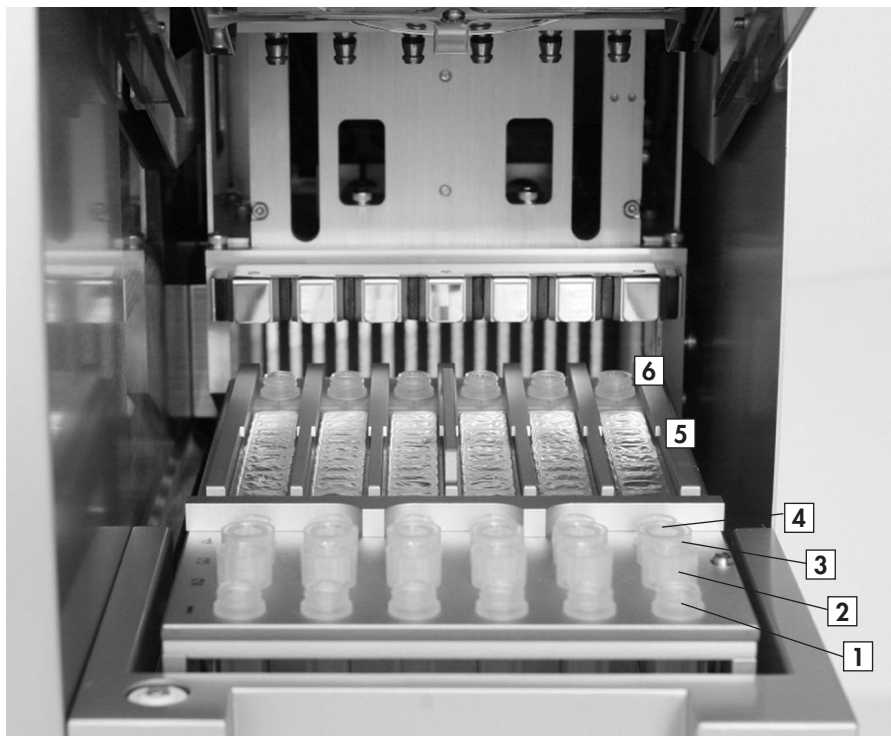


Figure 4. Typical EZ1 worktable.

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: Empty, unless option of 80% ethanol wash step is selected. If 80% ethanol wash step is selected, 2 ml tubes containing 1800 μ l 80% ethanol are loaded here.
4. Fourth row: Sample tubes (2 ml) are loaded here.
5. Reagent cartridges are loaded into the cartridge rack.
6. Heating block: this is empty for Blood Protocols.

Data tracking with the EZ1 Advanced and EZ1 Advanced XL

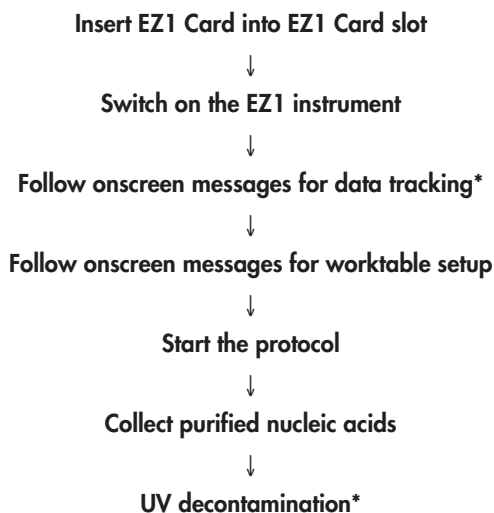
The EZ1 Advanced and EZ1 Advanced XL enable complete tracking of a variety of data for increased process control and reliability. The EZ1 Kit lot number and expiration dates are entered at the start of the protocol using the Q-Card bar code. A user ID and the Q-Card bar code can be entered manually via 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 each 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 9).

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 the report file is shown in Appendix B (page 28). 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



* 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 purification of DNA. Table 3 shows typical yields obtained from different sample volumes and sample types.

Table 3. DNA yields obtained from different sample types using EZ1 DNA procedures

Sample type	Sample amount	DNA yield
Blood*	200 µl	4–8 µg
Blood*	350 µl	5–12 µg
Buffy coat enriched >9x [†]	75 µl	6.5–14.5 µg
Buffy coat enriched ≤9x	150 µl	8–14 µg
Buffy coat with low leukocyte concentration	300 µl	Up to 14 µg

* Whole blood with $4\text{--}7 \times 10^6$ white blood cells/ml; elution volume 200 µl.

[†] Prepared from blood bag, 10x enrichment. This type of buffy coat preparation tends to result in very efficient leukocyte enrichment.

Protocol: Purification of DNA from Whole Blood

Select the appropriate EZ1 Kit and EZ1 Card according to the volume of your whole blood samples and the EZ1 instrument you are using.

Table 4. Selection of EZ1 Kit and EZ1 Card

Volume of blood sample	EZ1 Kit required	EZ1 Card required	Volume of eluted DNA
200 μ l	EZ1 DNA Blood 200 μ l Kit	EZ1 Advanced XL DNA Blood Card, [*] EZ1 Advanced DNA Blood Card, [†] or EZ1 DNA Blood Card [‡]	50 μ l, 100 μ l, or 200 μ l
350 μ l	EZ1 DNA Blood 350 μ l Kit	EZ1 Advanced XL DNA Blood Card, [*] EZ1 Advanced DNA Blood Card, [†] or EZ1 DNA Blood Card [‡]	50 μ l, 100 μ l, or 200 μ l

* EZ1 Advanced XL Cards are only for use with the EZ1 Advanced XL.

† EZ1 Advanced Cards are only for use with the EZ1 Advanced.

‡ EZ1 Cards are only for use with the BioRobot EZ1.

Important points before starting

- If using the EZ1 DNA Blood 200 μ l Kit or EZ1 DNA Blood 350 μ l Kit for the first time, read "Important Notes" (page 10).
- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In the case of liquid spillage, refer to "Safety Information" (page 6). Do not use damaged kit components, since their use may lead to poor kit performance.
- The protocols include an option to perform 80% ethanol washes instead of washes using the buffer provided in the reagent cartridge. This may be advantageous for some downstream applications. If this option is selected, 2 ml tubes containing 1800 μ l 80% ethanol should be placed in row 3 of the worktable by the user (see Figure 4, page 15). Follow the instructions given in the onscreen messages.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 6 for safety information.

- All steps of the protocol should be performed at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of two 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

- The buffer in well 1 of the reagent cartridge (i.e., 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 warming at 37°C and then place at room temperature (15–25°C).
- Equilibrate reagent cartridges to room temperature (15–25°C) before use.
- If using fresh blood, mix the blood samples thoroughly before loading them onto the EZ1 instrument to ensure homogeneity of the sample.
- If using frozen blood samples, thaw the blood samples and equilibrate to room temperature (15–25°C). Mix the blood samples thoroughly before loading them onto the EZ1 instrument to ensure homogeneity of the sample.

Procedure

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

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.

4. Press "1" or "2" to start worktable setup for the 200 µl Protocol or 350 µl Protocol, respectively.

Note: When using the data tracking option with the EZ1 Advanced XL, the display shows which kit has been selected according to the Q-Card scanned.

5. Choose the elution volume: press "1" to elute in 50 µl, "2" to elute in 100 µl, or "3" to elute in 200 µl.

6. Proceed through the text shown on the display.

The text summarizes the following steps which describe the loading of the worktable.

7. Open the instrument door.

8. Invert reagent cartridges 4 times 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.†
11. Load tip holders containing filter-tips into the second row.†
12. Load opened sample tubes containing 200 µl or 350 µl blood into the fourth row.†
13. Close the instrument door.
14. Press "START" to start the purification procedure.

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

The EZ1 Advanced and 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. Remove the elution tubes containing the purified DNA. Discard the sample-preparation waste.‡
18. ▲ **Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**
19. To run another protocol, press "ESC", prepare samples, 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.

* See Figure 3B on page 14.

† See Figure 4 on page 15.

‡ Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 6 for safety information.

Protocol: Purification of DNA from Buffy Coat

Select the appropriate EZ1 Kit and EZ1 Card according to the sample type and the EZ1 instrument you are using.

Table 5. Selection of EZ1 Kit and EZ1 Card

Volume of sample	EZ1 Kit required	EZ1 Card required	Volume of eluted DNA
75 µl buffy coat, enriched >9x or 150 µl buffy coat, enriched ≤9x or 300 µl buffy coat with low leukocyte concentration	EZ1 DNA Blood 350 µl Kit	EZ1 Advanced XL DNA Buffy Coat Card,* EZ1 Advanced DNA Buffy Coat Card, [†] or EZ1 DNA Buffy Coat Card [‡]	200 µl

* EZ1 Advanced XL Cards are only for use with the EZ1 Advanced XL.

[†] EZ1 Advanced Cards are only for use with the EZ1 Advanced.

[‡] EZ1 Cards are only for use with the BioRobot EZ1.

Important points before starting

- If using the EZ1 DNA Blood Kit or working with buffy coat for the first time, read “Important Notes” (page 10).
- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In the case of liquid spillage, refer to “Safety Information” (page 6). Do not use damaged kit components, since their use may lead to poor kit performance.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 6 for safety information.
- All steps of the protocol should be performed at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of two 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

- The buffer in well 1 of the reagent cartridge (i.e., 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 warming at 37°C and then place at room temperature (15–25°C).
- Equilibrate reagent cartridges to room temperature (15–25°C) before use.
- If using fresh buffy coat, mix the buffy coat samples thoroughly before loading them onto the EZ1 instrument to ensure homogeneity of the sample.
- If using frozen buffy coat samples, thaw the buffy coat samples and equilibrate to room temperature (15–25°C). Mix the buffy coat samples thoroughly before loading them onto the EZ1 instrument to ensure homogeneity of the sample.

Procedure

Preparation of buffy coat

1. **Centrifuge whole blood at 300 x g for 10 min at room temperature (15–25°C).**

Whole blood samples containing a standard anticoagulant (EDTA, citrate, or heparin) should be used.

After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat, containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes.

2. **Carefully transfer the middle layer containing the concentrated leukocytes to a new tube (not supplied). First pipet as much of the gray-white interface as possible followed by equal portions of the layers directly over and under the interface.**

In some cases it may be helpful to carefully aspirate off part of the plasma layer before harvesting the leukocytes.

A 1.8 ml whole blood sample should yield approximately 200 µl buffy coat.

Scaling up the preparation (e.g., to obtain 1 ml buffy from 9 ml whole blood) may improve the efficiency of the leukocyte harvest.

3. **Proceed with DNA purification immediately or store samples at –20°C for purification at a later date.**

DNA purification

4. **Insert ▲ the EZ1 Advanced DNA Buffy Coat Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Buffy Coat Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Buffy Coat Card completely into the EZ1 Card slot of the BioRobot EZ1.**

5. Switch on the EZ1 instrument.
6. Press "START" to start protocol setup. ▲ Follow the onscreen instructions for data tracking.

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.

7. Depending on the sample volume used in step 1, press "1" for 75 µl sample volume, press "2" for 150 µl sample volume, or press "3" for 300 µl sample volume.

8. Press any key to proceed through the text shown on the display.

The text summarizes the following steps which describe the loading of the worktable.

9. Open the instrument door.

10. Invert reagent cartridges 4 times 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.

11. 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.

12. Load opened elution tubes into the first row.†

13. Load tip holders containing filter-tips into the second row.†

14. Load opened sample tubes containing buffy coat samples into the fourth row.‡

Note: The buffy coat samples should be 75–300 µl depending on the type of sample used (see Table 5, page 21).

15. Close the instrument door.

16. Press "START" to start the purification procedure.

The automated purification procedure takes approximately 20 min.

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

The EZ1 Advanced and 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.

18. Open the instrument door.

19. Remove the elution tubes containing the purified DNA. Discard the sample-preparation waste.‡

* See Figure 3B on page 14.

† See Figure 4 on page 15.

‡ Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 6 for safety information.

20. ▲ **Optional: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.**
21. **To run another protocol, press “ESC”, prepare samples, and follow the procedure from step 7 onward. Otherwise, press “STOP” twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.**
22. **Clean the EZ1 instrument.**

Follow the maintenance instructions in the user manual.

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 or 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) Varying pipetting volumes
To ensure pipetting accuracy, it is important to have correct buffer volumes in the reagent cartridges and optimal fit of the filter tips 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.
- d) Frozen blood or buffy coat samples not mixed properly after thawing
Thaw frozen blood or buffy coat samples at room temperature (15–25°C) with mild agitation to ensure thorough mixing.

DNA does not perform well in downstream applications

- a) Insufficient DNA used in downstream application
Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see “Quantification of DNA”, page 27).
- b) Excess DNA used in downstream application
Excess DNA can inhibit some enzymatic reactions. Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see “Quantification of DNA”, page 27).
- c) Inhibition of downstream application
Some downstream applications may show superior performance if an 80% ethanol wash is performed instead of washes using buffers in the reagent cartridges (see page 18).

Low A_{260}/A_{280} ratio for purified nucleic acids

Absorbance reading at 320 nm not subtracted from the absorbance readings obtained at 260 nm and 280 nm
To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see “Quantification of DNA”, page 27).

Low DNA yield from buffy coat

- a) Clogging due to sample overload
Reduce the amount of sample. The maximum recommended amount of cells to use as starting material is 5×10^6 .
- b) Poor buffy coat preparation
Ensure that the leukocyte fraction is harvested efficiently.
- c) Low leukocyte count in the whole blood sample
Increase whole blood amount and keep the volume of leukocytes harvested constant.

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

Storage of DNA

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

Quantification of DNA

The concentration of DNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. An absorbance of 1 unit at 260 nm corresponds to 50 µg of DNA per ml ($A_{260}=1 \rightarrow 50 \mu\text{g/ml}$). Use buffer of neutral pH (e.g., 10 mM Tris·Cl, * pH 7.0) to dilute the samples and to calibrate the spectrophotometer.† Carryover of magnetic particles in the eluate may affect the A_{260} reading, but should not affect the performance of the DNA in downstream applications. If the purified DNA is to be analyzed by fluorescent capillary sequencing, the tube containing the eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see below).

To quantify DNA isolated using the EZ1 system:

- Apply the tube containing the DNA to a suitable magnetic separator (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912) for 1 minute. If a suitable magnetic separator is not available, centrifuge the tube containing the DNA for 1 minute at full speed in a microcentrifuge to pellet any remaining magnetic particles.
- Once separation is complete, carefully withdraw 10–50 µl of isolated DNA and dilute to a final volume of 100 µl in buffer of neutral pH.
- Measure the absorbance at 320 nm and 260 nm. Subtract the absorbance reading obtained at 320 nm from the reading obtained at 260 nm to correct for the presence of magnetic particles.

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

Total amount of DNA isolated = concentration \times volume of sample in ml

Purity of DNA

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

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

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

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 Blood 200

Date of run _____ Apr 21, 2008
Start of run: _____ 12:57
End of run: _____ 13:17
Status run: _____ o.k.
Error Code: _____
Sample input Vol [ul]: _____ 200
Elution volume [ul]: _____ 100

Channel A:

Sample ID: _____ 123456789
Reagent Kit number: _____ 9801601
Reagent Lot number: _____ 23456789
Reagent Expiry date: _____ Jun 14, 2009
Assay kit ID: _____ 848373922
Note: _____ 2000

Channel B:

Sample ID: _____ 234567890
Reagent Kit number: _____ 9801601
Reagent Lot number: _____ 23456789
Reagent Expiry date: _____ Jun 14, 2009
Assay kit ID: _____ 836266738
Note: _____

Channel C:

Sample ID: _____ 345678901
Reagent Kit number: _____ 9801601
Reagent Lot number: _____ 23456789
Reagent Expiry date: _____ Jun 14, 2009
Assay kit ID: _____ 883727832
Notes: _____ 1000

Channel D:

Sample ID: _____ 456789012
Reagent Kit number: _____ 9801601
Reagent Lot number: _____ 23456789
Reagent Expiry date: _____ Jun 14, 2009
Assay kit ID: _____ 763684837
Note: _____

Channel E:

Sample ID: _____ 567890123
Reagent Kit number: _____ 9801601
Reagent Lot number: _____ 23456789
Reagent Expiry date: _____ Jun 14, 2009
Assay kit ID: _____ 4387728002
Note: _____

Channel F:

Sample ID: _____ 678901234
Reagent Kit number: _____ 9801601
Reagent Lot number: _____ 23456789
Reagent Expiry date: _____ Jun 14, 2009
Assay kit ID: _____ 509389403
Note: _____ 50

Ordering Information

Product	Contents	Cat. no.
EZ1 DNA Blood 200 µl Kit (48)	48 Reagent Cartridges (Blood 200 µl), 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes (2 ml), 50 Elution Tubes (1.5 ml)	951034
EZ1 DNA Blood 350 µl Kit (48)	48 Reagent Cartridges (Blood 350 µl), 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes (2 ml), 50 Elution Tubes (1.5 ml)	951054
EZ1 Advanced	Robotic instrument for automated purification of nucleic acids from up to 6 samples using EZ1 Kits, 1-year warranty on parts and labor*	9001410
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
Accessories		
EZ1 Advanced DNA Blood Card	Preprogrammed card for EZ1 Advanced DNA blood protocols	9018293
EZ1 Advanced XL DNA Blood Card	Preprogrammed card for EZ1 Advanced XL DNA blood protocols	9018695
EZ1 Advanced DNA Buffy Coat Card	Preprogrammed card for EZ1 Advanced DNA buffy coat protocols	9018294
EZ1 Advanced XL DNA Buffy Coat Card	Preprogrammed card for EZ1 Advanced XL DNA buffy coat protocols	9018697

* 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.
Related products		
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
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

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Visit www.qiagen.com/goto/EZ1Advanced to find out more about other EZ1 Kits!

Notes

Notes

Notes

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