May 2020

EZ1® DNA Blood Handbook

For automated purification of DNA from blood and buffy coat using EZ1 instruments

Sample to Insight

QIAGEN

Contents

Kit Contents

EZ1 DNA Blood Kits*	**200 µl**	**350 µl**
Catalog no.	**951034**	**951054**
No. of preps	**48**	**48**
Reagent Cartridge, Blood 200 µl (1023745)	48	–
Reagent Cartridge, Blood 350 µl (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
Quick-Start Protocol	1	1

* For details about the EZ1 Cards to be used with these kits, see Table 2, page 14, 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.

Shipping and 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.

Intended Use

The EZ1 DNA Blood 200 µl Kit and EZ1 DNA Blood 350 µl 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.

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 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, refer to the instrument user manual for decontamination instructions.

Quality Control

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

Introduction

The EZ1 DNA Blood 200 µl Kit and EZ1 DNA Blood 350 µl 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. If using BioRobot® EZ1 and EZ1 Advanced instruments, up to 6 samples are processed in a single run. With the EZ1 Advanced XL, up to 14 samples 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 (Figure 1, page 7). 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.

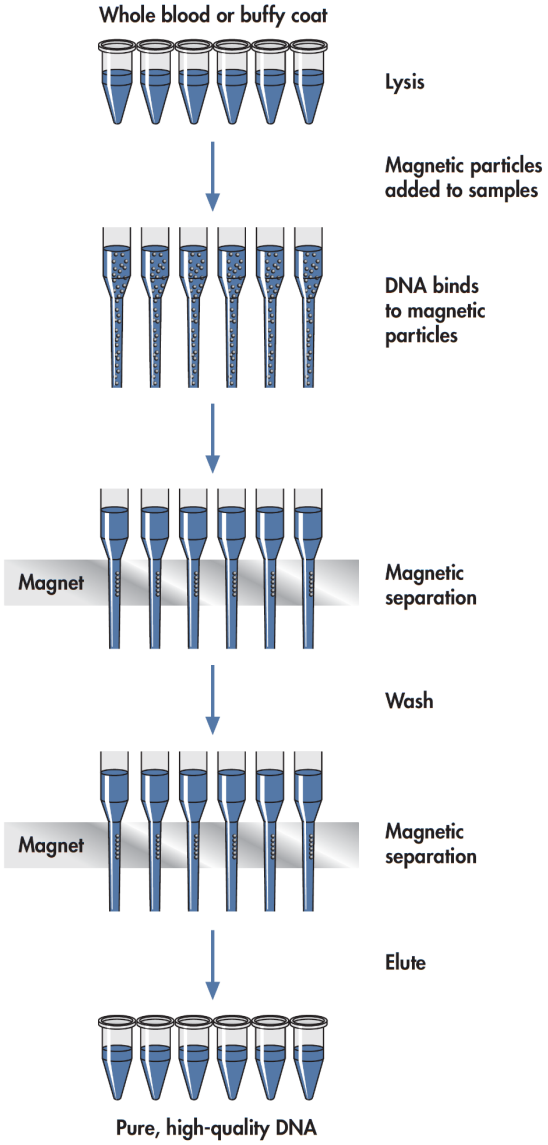

Figure 1. EZ1 DNA extraction procedure for whole blood/buffy coat.

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

- Soft paper tissue
- Water
- 70% ethanol
- Pipettes
- Thermomixer, heated orbital incubator, heating block, or water bath

For users of BioRobot EZ1

- EZ1 DNA Blood Card (cat. no. 9015585; see Table 2, page 14)

For users of EZ1 Advanced

- EZ1 Advanced DNA Blood Card (cat. no. 9018293; see Table 2, page 14)

For users of EZ1 Advanced XL (cat. no. 9001492 or 9001874)

- EZ1 Advanced XL DNA Blood Card (cat. no. 9018695; see Table 2, page 14)

For EZ1 Advanced and EZ1 Advanced XL users

For documentation purposes

- EZ1 Advanced Communicator software (supplied with the EZ1 Advanced and the EZ1 Advanced XL)

For Blood Protocol

- **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 µl or 350 µl	50 µl, 100 µl, or 200 µl
Buffy coat*		
Buffy coat, enriched >9x†	50–75 µl	200 µl
Buffy coat, enriched 9x‡	100–150 µl	200 µl
Buffy coat with low leukocyte concentration§	200–300 µl	200 µl

* For each buffy coat protocol, the maximum number of cells to use as starting material is 5 x 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. In general, follow instructions and guidelines of the blood collection tube provider.

Frozen samples should be thawed at room temperature 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.

¶ 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.

- 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, because 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 to –80°C.

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. 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" in Appendix A (page 29), 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 instance, 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 2). The availability of various protocols increases the flexibility of EZ1 instruments.

Figure 2. Ease of protocol setup using EZ1 Cards. Inserting an EZ1 Card that contains 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 3); otherwise, essential instrument data could be lost, leading to a memory error. Make sure to turn off the instrument before removing or replacing the EZ1 Cards; otherwise, a memory error can occur.

Figure 3. 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/resources** 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 4). Each well of the cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Because 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.

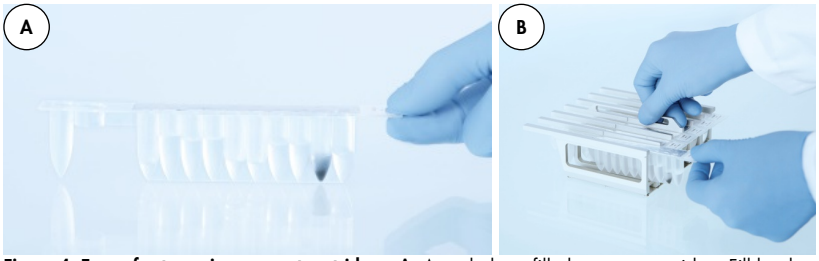

Figure 4. Ease of setup using reagent cartridges. A: A sealed, prefilled reagent cartridge. Fill levels vary, depending on the type of reagent cartridge. **B**: Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded.

Worktable

The worktable of EZ1 instruments is where the user loads samples and the components of the EZ1 Kits (Figure 5).

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.

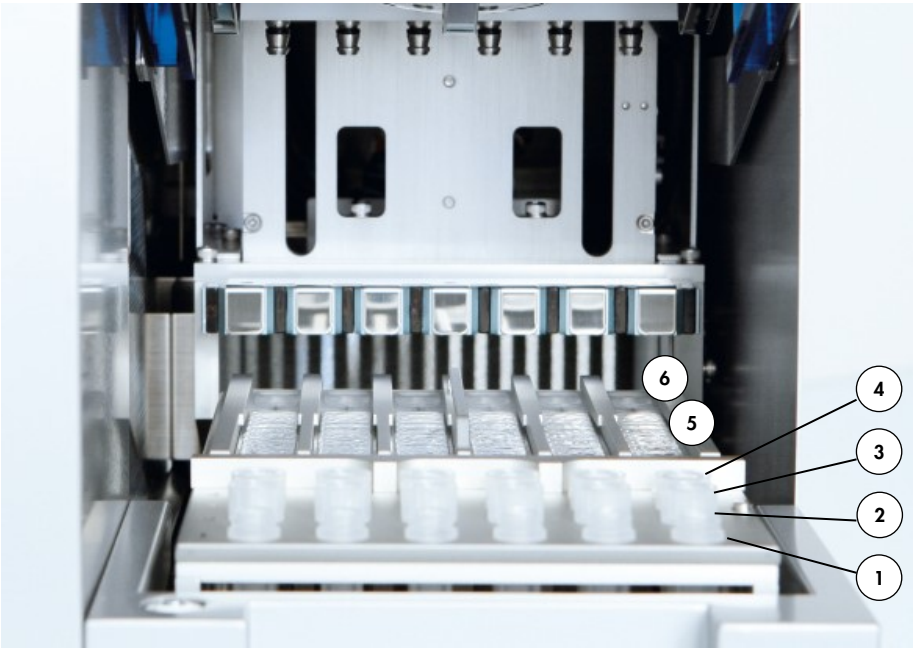

Figure 5. The EZ1 Advanced worktable. The EZ1 Advanced has 6 columns for the simultaneous processing of 6 samples, while the EZ1 Advanced XL has 14 columns and can process 14 samples at the same time. **1**: First row: Elution tubes (1.5 ml) are loaded here. **2**: Second row: Tip holders containing 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 µl 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 8).

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 31). In report files, the 6 pipetting channels of the EZ1 Advanced are named, from left to right, channels A–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 *EZ1 Advanced User Manual*, **www.qiagen.com/HB-0111**, or the *EZ1 Advanced XL User Manual*, **www.qiagen.com/HB-0176**.

Workflow of EZ1 operation

Insert EZ1 Card into EZ1 Card slot

↓

Switch on the EZ1 instrument

↓

Follow onscreen messages for data tracking*

↓

Follow onscreen messages for worktable setup

↓

Start the protocol

↓

Collect purified nucleic acids

↓

UV decontamination*

* EZ1 Advanced and EZ1 Advanced XL only.

Yield of purified DNA

DNA yields depend on the sample type, number of nucleated cells in the sample, and the protocol used for 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 4–7 x 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

Blood sample volume	EZ1 Kit required	EZ1 Card required	Volume of eluted DNA
200 µl	EZ1 DNA Blood 200 µl Kit	EZ1 Advanced XL DNA Blood Card,* EZ1 Advanced DNA Blood Card,† or EZ1 DNA Blood Card‡	50 µl, 100 µl, or 200 µl
350 µl	EZ1 DNA Blood 350 µl Kit	EZ1 Advanced XL DNA Blood Card,* EZ1 Advanced DNA Blood Card,† or EZ1 DNA Blood Card‡	50 µl, 100 µl, or 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 200 µl Kit or EZ1 DNA Blood 350 µl 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 case of liquid spillage, refer to "Safety Information" (page **Error! Bookmark not defined.**). Do not use damaged kit components, because 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 µl 80% ethanol should be placed in row 3 of the worktable by the user (see Figure 5, 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 **Error! Bookmark not defined.** for "Safety Information".
- All steps of the protocol should be performed at room temperature. During the setup procedure, work quickly.
- In some steps of the procedure, 1 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

- 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.
  Important: Do not vigorously shake the cartridge, because that will lead to foaming of the buffer.
- Equilibrate reagent cartridges to room temperature 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. 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 steps for loading the worktable.

7. Open the instrument door.

8. Gently invert reagent cartridges 4 times to mix the magnetic particles. And 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 on the cartridge until it clicks into place.

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

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

12. **Optional**: If an additional wash step shall be done, load 2 ml tubes containing 1800 µl 80% ethanol in the third row.†

13. Load the opened sample tubes containing 200 µl or 350 µl blood into the fourth row.†

14. Close the instrument door.

* See Figure 4B on page 14.

† See Figure 5 on page 15.

15. Press **Start** to begin the purification procedure.

    The automated purification procedure takes approximately 20 min.

16. 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.

17. Open the instrument door.

18. Remove the elution tubes containing the purified DNA. Discard the sample-preparation waste.*

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

20. 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.

21. Clean the EZ1 instrument.

    Follow the maintenance instructions in the user manual.

* Sample waste contains guanidine salts and is therefore not compatible with bleach. See "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 case of liquid spillage, refer to "Safety Information" (page **Error! Bookmark not defined.**). 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 **Error! Bookmark not defined.** for "Safety Information".
- All steps of the protocol should be performed at room temperature. During the setup procedure, work quickly.
- In some steps of the procedure, 1 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

- 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.
  Important: Do not vigorously shake the cartridge, because that will lead to foaming of the buffer.
- Equilibrate reagent cartridges to room temperature 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. 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.

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

   After centrifugation, 3 different layers are visible: the clear upper 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 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 –30 to –15°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 steps for loading 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 on the cartridge until it clicks into place.

12. Load the opened elution tubes into the first row.†
13. Load the 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 22).

15. Close the instrument door.
16. Press **Start** to begin 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.‡
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.

* See Figure 4B on page 14.

† See Figure 5 on page 15.

‡ Sample waste contains guanidine salts and is therefore not compatible with bleach. See page 4 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 in 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 or protocols in this handbook (for contact information, visit **support.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 to confirm that the printer is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port. Check to confirm that the serial port is set for use with a printer.
c) Report file not sent to the PC	Check to confirm that the PC is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port. Check to confirm that 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 or dirty filter tips	
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.
c) Varying pipetting volumes	To ensure pipetting accuracy, ensure that samples are thoroughly mixed and that reagent cartridges have not passed their expiry date, as the latter can result in the loss of fluids. Do not use cartridges with visible foam. Also, perform regular maintenance as described in the instrument user manual, because greasing the O-rings of the dilutors affects the fit of the filter tips.

		Comments and suggestions
d)	Frozen blood or buffy coat samples not mixed properly after thawing	Thaw frozen blood or buffy coat samples at room temperature, with mild agitation to ensure thorough mixing.
e)	Blood sample is clotted	Ensure that the tubes used to collect and store whole blood contain EDTA, ACD, or heparin as anticoagulant. Follow the instructions of the collection tube provider. Fresh blood can be stored at 2–8°C for up to 10 days. Frozen blood should be in tubes containing EDTA. It can keep for more than 10 days if stored at –70 to –80°C.
f)	Beads transgressed the filter	Dilutor may be impaired. Contact field service through **support.qiagen.com**.
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", Appendix A, page 29).
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", Appendix A, page 29).
c)	Inhibition of downstream application	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).
(d)	Poor storage conditions led to poor-quality DNA	For storage of up to 10 days, collect blood in tubes with EDTA as 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, because low levels of DNA degradation will occur after this time. For storage longer than 10 days, collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store the tubes at –70 to –80°C. Do not use blood that shows signs of coagulation. Fresher blood samples may yield better results. Follow the collection tube provider's instructions.
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", Appendix A, page 29).

		Comments and suggestions
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 x 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 h, or at –30 to –15°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 → 50 µg/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 min. If a suitable magnetic separator is not available, centrifuge the tube containing the DNA for 1 min 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.

* 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.

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

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

Concentration of DNA sample = 50 µg/ml x (A_{260} – A_{320}) x dilution factor
Total amount of DNA isolated = concentration x 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.*

* 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 XL, System	Robotic instrument for automated purification of nucleic acids from up to 14 samples using EZ1 Kits, installation, training, and 1-year warranty on parts and labor	9001874
EZ1 Advanced XL, Priority	Robotic workstation for automated purification of nucleic acids from up to 14 samples using EZ1 Kits: includes Priority Package with software, installation, training, 2-year warranty on parts and labor, and 2 preventive maintenance visits	9001875
EZ1 Advanced XL, Priority Package Plus	Robotic workstation for automated purification of nucleic acids from up to 14 samples using EZ1 Kits: includes Priority Package with software, installation, training, 3-year warranty	9001876

Product	Contents	Cat. no.
	on parts and labor, and 3 preventive maintenance visits	
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
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

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

Document Revision History

Date	Changes
05/2020	Corrected image in Figure 1. Replaced handbook with quick-start protocol in "Kit Contents". Renamed "Warnings and Precautions" to "Safety Information" and removed instructions to use sodium hypochlorite for instrument decontamination, as the user manual warns against this. Added statement that the EZ1 Advanced XL can process up to 14 samples. Removed RNase-free pipette tips (not needed), PC, printer, monitor, and all discontinued products from "Equipment and Reagents to Be Supplied by User". Replaced images with updated versions. Added warning in protocols to not shake the reagent cartridge vigorously, to prevent foaming of the buffer. Added optional wash step in "Protocol: Purification of DNA from Whole Blood". Added troubleshooting information for low DNA yield and dirty filter tips. Replaced precise storage temperatures with temperature ranges. Removed sentence referencing broken link, www.qiagen.com/goto/EZ1Advanced.

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HB-0197-004 05/2020