June 2020

EZ1® ccfDNA Handbook

For semi-automated concentration and purification of circulating cell-free DNA (ccfDNA) from plasma or serum using the EZ1 Advanced XL instrument

Sample to Insight

QIAGEN

Contents

Kit Contents

EZ1 ccfDNA Mini Kit	**(48)**
Catalog no.	**954134**
Number of preps	**48***
Proteinase K	1 x 10 ml
Bead Binding Buffer	1 x 20 ml
Magnetic Bead Suspension	2 x 1.6 ml
Bead Elution Buffer	1 x 12 ml
Bead Elution Tubes	50
Elution Tubes 1.5 ml	100
Reagent Cartridges, EZ1 ccfDNA†‡	48
Disposable Tip Holders	50
Disposable Filter-Tips 50	50
Q-Card§	1
Quick-Start Protocol	1

* Forty-eight preps is guaranteed for 1–2 ml plasma/serum. If 4 ml plasma is used, the number of preps is 24.

† Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for safety information.

‡ Contains sodium azide as a preservative.

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

EZ1 ccfDNA Midi Kit	**(48)**
Catalog no.	**954154**
Number of preps	**48***
Proteinase K	2 x 10 ml
Bead Binding Buffer	2 x 20 ml
Magnetic Bead Suspension	5 x 1.6 ml
Bead Elution Buffer	1 x 12 ml
Bead Elution Tubes	50
Elution Tubes 1.5 ml	100
Reagent Cartridges, EZ1 ccfDNA†‡	48
Disposable Tip Holders	50
Disposable Filter-Tips 50	50
Q-Card§	1
Quick-Start Protocol	1

* Forty-eight preps is guaranteed for 4–5 ml plasma/serum. If 10 ml plasma is used, the number of preps is 24.

† Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for safety information.

‡ Contains sodium azide as a preservative.

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

Shipping and Storage

EZ1 ccfDNA Mini and Midi Kits are shipped at ambient temperature. Magnetic Bead Suspension should be stored at 2–8°C upon arrival. All other buffers and reagents can be stored at room temperature (15–25°C). Do not freeze the reagent cartridges. When stored properly, buffers and reagent cartridges are stable until the kit expiration date.

EZ1 ccfDNA Mini and Midi Kits contain a ready-to-use Proteinase K solution, which is dissolved in a specially formulated storage buffer. The Proteinase K is stable for up to 1 year after delivery when stored at room temperature, unless otherwise indicated on the label. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

Intended Use

EZ1 ccfDNA Mini and Midi Kits are 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 these 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.

Quality Control

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

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.

Some buffers in the reagent cartridges contain guanidine hydrochloride or 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 Advanced XL, please refer to the instrument user manual for decontamination instructions.

Introduction

Free-circulating DNA, such as tumor-specific extracellular DNA fragments and mRNAs in the blood or fetal DNA in maternal blood, are present in serum or plasma usually as short fragments, <1000 bp (DNA) or <1000 nt (RNA).

EZ1 ccfDNA Kits enable efficient purification of these circulating DNAs from human plasma or serum samples. These samples can be either fresh or frozen (provided that they have not been frozen and thawed more than once). Human serum or plasma samples can be generated using blood collection tubes such as the PAXgene® Blood ccfDNA Tube. The initial step of enriching ccfDNA on magnetic beads facilitate the flexible and scalable handling of sample volumes from 1 to 10 ml. Subsequent ccfDNA cleanup on the EZ1 Advanced XL instrument provides convenient automated bead-based purification of up to 14 samples in parallel.

Free-circulating DNA is eluted in Buffer AVE, ready for use in downstream reactions or storage at –30 to –15°C. Purified nucleic acids are free of proteins, nucleases, and other impurities.

Principle and workflow

EZ1 ccfDNA Kits use a fast procedure involving manual pre-enrichment of ccfDNA onto magnetic beads, followed by automated cleanup on the EZ1 Advanced XL. The magnetic-particle technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles. The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples.

The simple procedure, which is suited for simultaneous processing of multiple samples, provides pure nucleic acids in less than 1 hour for 14 samples.

Sample volumes

The EZ1 ccfDNA Mini Kit has been optimized for sample volumes 1–4 ml, while the EZ1 ccfDNA Midi Kit has been optimized for sample volumes 4–10 ml. Yield depends on the sample volume and the concentration of circulating nucleic acids in the sample (typically, 1–100 ng/ml in plasma).

Sample numbers

EZ1 ccfDNA Kits have been designed to be flexible with serum or plasma input volumes, and the bead-binding procedure to generate the pre-eluate can be scaled as desired. Each kit has been designed with a specific target volume in mind: the Mini kit is designed to process 48 1–2 ml serum or plasma samples, while the Midi kit has been designed to process 48 4–5 ml serum or plasma samples. Processing larger volumes is possible with these kits, but this will directly reduce the number of preparations that can be performed with 1 kit. For instance, the Mini Kit can be scaled to 4 ml plasma samples, but then the kit will contain only enough bead-binding and elution reagents for 24 samples in total. Likewise, the Midi Kit can be scaled to 10 ml plasma samples, but then will only have sufficient reagents to process 24 samples. By designing the kits to adapt to the most commonly required scenarios, we are able to keep prices low and minimize waste. If you need to regularly process volumes of 10 ml or higher, please contact QIAGEN Technical Services at **support.qiagen.com** for advice.

Procedure

Preparing plasma from whole blood

To isolate circulating, cell-free nucleic acids from blood samples, please refer to "Appendix A: Recommendations for Plasma Separation and Storage", page 26. This protocol includes a high *g*-force centrifugation step to remove cellular debris and reduce the amount of cellular or genomic DNA and RNA in the sample.

Manual pre-enrichment

Lysing samples and binding to beads

Up to 10 ml of plasma or serum can be processed in a standard 15 ml centrifuge tube. Bead-binding buffer, Magnetic Bead Suspension, and Proteinase K are added to the sample in the appropriate ratio to the sample volume. Circulating DNA is bound to magnetic beads during end-over-end rotation, during which the samples are lysed at room temperature in the presence of Proteinase K, which ensures complete release of nucleic acids from bound proteins.

Bead collection and elution

Magnetic beads with bound DNA are collected in a pellet on a magnet rack, and the supernatant is discarded. The bound DNA is then eluted from the beads in a special buffer, after which the beads (now with no DNA bound to them) are separated from the pre-eluate containing the DNA.

Automated ccfDNA cleanup on the EZ1 Advanced XL Instrument

Adsorption to silica-coated magnetic particles

Binding buffer is added to the pre-eluate to adjust binding conditions. Samples are thoroughly mixed with magnetic particles to allow adsorption of ccfDNA to the silica surface. Salt and pH conditions ensure that proteins and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not bound to the magnetic particles.

Washing of bound nucleic acids

While ccfDNA remains bound to the magnetic particles, contaminants are efficiently washed away during a series of wash steps using first wash buffer 1, and then wash buffer 2, and then ethanol.

Elution of purified nucleic acids

Highly purified ccfDNA is eluted in a single step in Buffer AVE (the eluate volume can range from 55 to 65 µl) and collected in 1.5 ml microcentrifuge tubes. The purified ccfDNA can be either used immediately in downstream applications or stored for future use.

We recommend storing purified circulating nucleic acids at 2–8°C if only for up to 24 hours or at –30 to –15°C if longer than 24 hours.

Yield and size of nucleic acids

Yields of free-circulating nucleic acids isolated from biological samples are normally well below 1 µg and are therefore difficult to determine with a spectrophotometer. Additionally, the small size of circulating DNA (main peak, approximately 160–180 bp) renders many fluorometric methods unreliable for yield determination. Quantitative amplification methods, such as real-time PCR, are recommended for determination of yields.

Size distribution of circulating nucleic acids purified using this procedure can be checked by analysis on an Agilent® Bioanalyzer®, TapeStation®, or a similar device. Alternatively, agarose gel electrophoresis and hybridization to a target-specific labeled probe is an option (Sambrook, J. and Russell, D.W. [2001] *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

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.

In addition to the EZ1 ccfDNA Mini or Midi Kit, the following supplies are required.

- EZ1 Advanced XL instrument (cat. no. 9001874)
- EZ1 Advanced XL ccfDNA Mini/Midi Card (cat. no. 9025647)
- Shaker for microcentrifuge tubes
- End-over-end shaker/rotator
- Magnet rack for 15 ml tubes, e.g., 15 ml/50 ml Tube Magnet (cat. no. 36935), AdnaMag-L (cat. no. 399921), or equivalent)
- Magnet rack for 2 ml tubes, e.g., 12-Tube Magnet (cat. no. 36912), AdnaMag-S (cat. no. 399911), or equivalent
- Pipettes (adjustable)
- Sterile pipette tips (pipette tips with aerosol barriers are recommended to help prevent cross-contamination)
- 15 ml centrifuge tubes

Important Notes

Working with the EZ1 Advanced XL instrument

The main features of the EZ1 Advanced XL Instrument include:

- Purification of high-quality nucleic acids from 1–14 samples per run
- Small footprint to save laboratory space
- Preprogrammed EZ1 Advanced XL Cards containing ready-to-use protocols for nucleic acid purification
- Prefilled, sealed reagent cartridges for easy, safe, and fast setup of the EZ1 Advanced XL Instrument

Additional features of 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 decontamination of the worktable surfaces

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

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 Instrument, and the instrument is then ready to run a protocol (Figure 1 and Figure 2). The availability of various protocols increases the flexibility of EZ1 instruments.

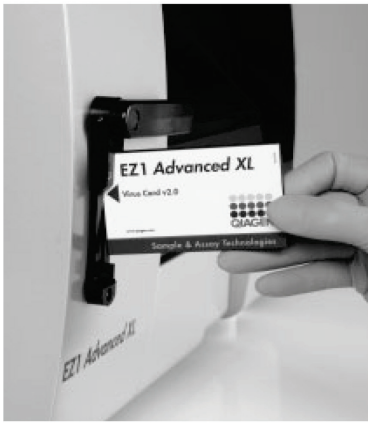


Figure 1. Ease of protocol setup using EZ1 Advanced XL Cards. Inserting an EZ1 Card, which contains a protocol, into the EZ1 Advanced XL instrument.

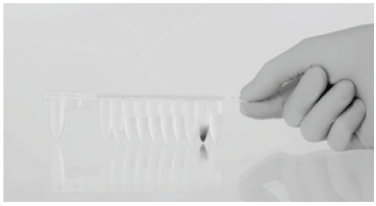
Figure 2. EZ1 Card completely inserted into EZ1 Card slot.

The instrument 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. Make sure to turn off the instrument before removing or replacing the EZ1 Cards; otherwise, a memory error can occur.

Reagent cartridges

Reagents for 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, binding buffer, wash buffer or elution buffer (Buffer AVE). Since each well contains only the required amount of reagent, generation of additional waste due to leftover reagent at the end of the purification procedure is avoided.

A



B

Figure 3. Ease of instrument setup using reagent cartridges. A: A sealed, prefilled reagent cartridge of the EZ1 ccfDNA Mini or Midi Kit. **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 the EZ1 Advanced XL instrument is where the user loads samples and the components of the EZ1 ccfDNA Mini/Midi Kits (Figure 4).

Details on worktable setup are provided in the protocol in this handbook and are also displayed in the vacuum fluorescent display (VFD) of the EZ1 Advanced XL control panel when the user starts worktable setup. The display also shows protocol status during the automated purification procedure.

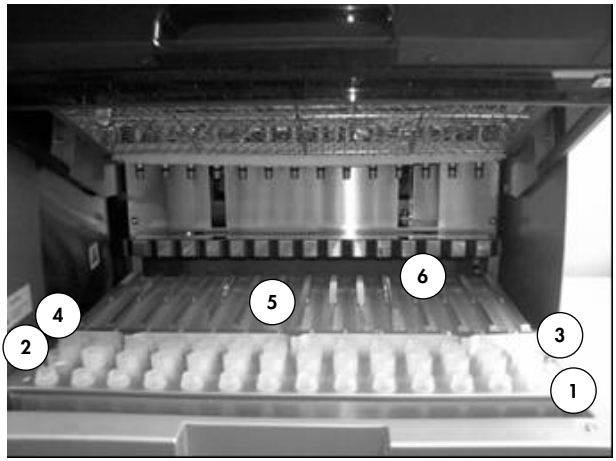


Figure 4. Worktable of an EZ1 Advanced XL Instrument.

1 First row: Elution tubes (ET) (1.5 ml) are loaded here.

2 Second row: Disposable tip holders (DTH) containing disposable filter-tips (DFT) are loaded here.

3 Third row: For the EZ1 ccfDNA Mini/Midi Kit protocol this row is empty.

4 Fourth row: Elution tubes (ET) (1.5 ml) containing the pre-eluate from manual ccfDNA enrichment are loaded here for the EZ1 ccfDNA Mini/Midi Kit protocol.

5 Reagent cartridges (RCB) loaded into the cartridge rack.

6 The heating block is empty for the EZ1 ccfDNA Mini/Midi Kit protocol.

Data tracking with the EZ1 Advanced XL

The EZ1 Advanced XL enables 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 using 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 XL can store up to 10 report files, and the data can be transferred to a PC or directly printed using a printer.

To receive report files on a PC, the EZ1 Advanced Communicator software needs to be installed. The software receives the report file and stores it in a folder that you define. After the PC has received the report file, you can use and process the file with a LIMS (Laboratory Information Management System) or other programs. An example of a report file is shown in "Appendix B: Example of an EZ1 Advanced Report File", page 28. In report files, 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 XL User Manual*, **www.qiagen.com/HB-0176**.

Workflow of EZ1 Advanced XL ccfDNA Mini/Midi operation

Insert EZ1 Card into the EZ1 Card slot

↓

Switch on the EZ1 instrument

↓

Follow onscreen messages for data tracking

↓

Follow onscreen messages for worktable setup

↓

Start the protocol

↓

Collect purified nucleic acids

↓

UV decontamination

Protocol: Purification of Circulating DNA from 1–4 ml Serum or Plasma Using the EZ1 ccfDNA Mini Kit

Important points before starting

- If using the EZ1 ccfDNA Mini Kit for the first time, read "Important Notes", page 12.
- 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 6. Do not use damaged kit components, because their use may lead to poor kit performance or contamination of the EZ1 instrument.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See "Safety Information", page 6.
- Perform all steps of the protocol at room temperature. During the setup procedure, work quickly.

Things to do before starting

- Prepare a shaker for microcentrifuge tubes at room temperature for use in step 4.
- Ensure availability of magnetic racks for 15 ml and 2 ml tubes.
- EZ1 instruments should only be switched on after an EZ1 card is inserted. Make sure that the EZ1 Card is completely inserted; 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.
- 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.

- Resuspend Magnetic Bead Suspension by pulse-vortexing for 1 min. Do not let the suspension settle for more than 2 min. Pipet from the center of the suspension.
- Before loading reagent cartridges into the EZ1 instrument, invert them 3 times to mix the magnetic particles, and then tap to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.

Procedure

1. Mix components according to Table 1 in a 15 ml tube (not provided), and incubate for 10 min at room temperature while shaking (slow speed) end-over-end.

Table 1. Component mix (EZ1 ccfDNA Mini Kit)

Plasma (ml)	Magnetic Bead Suspension (µl)	Proteinase K (µl)	Bead Binding Buffer (µl)
1	30	55	150
2	60	110	300
3	90	165	450
4	120	220	600

2. Spin briefly (30 s at 200 x *g*) to remove any solution in cap.
3. Place the tube containing bead solution into a magnetic rack for 15 ml tubes. Let stand for at least 1 min until the solution is clear. Discard supernatant.
4. Remove the tube from the magnetic rack and add 200 µl Bead Elution Buffer to the bead pellet. Vortex to resuspend beads, and pipet up and down to mix and rinse residual beads from the tube wall. Transfer the mixture (including beads) into a Bead Elution Tube. Incubate for 5 min on a shaker for microcentrifuge tubes at room temperature and 300 rpm.

5. Place the Bead Elution Tube containing the bead solution into a magnetic rack for 2 ml tubes. Let stand for at least 1 min, until the solution is clear. Transfer supernatant (pre-eluate) to a new 1.5 ml elution tube and save for use in step 7.

   Note: Avoid transferring any magnetic beads in this step. Carryover may result in reduced ccfDNA yield.

   Note: Each sample requires two 1.5 ml elution tubes: 1 for loading the pre-eluate on the EZ1 instrument and 1 to collect ccfDNA after purification (worktable setup in step 7 will guide you).

6. Insert the EZ1 Advanced XL ccfDNA Mini/Midi Card completely into EZ1 Advanced XL Card slot of the EZ1 Advanced XL Instrument. Switch on the instrument.
7. Press **Start** to start worktable setup of the EZ1 ccfDNA Mini protocol. Open the instrument door. Follow onscreen instructions for worktable setup and data tracking. Close the door, and press **Start** to start the protocol.
8. The display shows "Protocol finished" when finished. Press **Esc**.
9. Open the instrument door. Remove elution tubes containing purified ccfDNA (in 60 µl) from the first row. Discard the sample preparation waste.* Press **Ent**. The report file is automatically transferred.

   Optional: Follow onscreen instructions for UV decontamination of table surfaces.

10. Perform regular maintenance after each run. Press **Esc** to return to the main menu.

    Note: Regular maintenance consists of cleaning the piercing unit and the worktable surfaces.

    Important: The piercing unit is sharp. The use of double gloves is recommended.

11. To run another protocol, press **Start** and follow the protocol from step 7. Otherwise, close the instrument door and switch off the instrument.

* Sample waste contains guanidine salts and is not compatible with bleach.

Protocol: Purification of Circulating DNA from 4–10 ml Serum or Plasma Using the EZ1 ccfDNA Midi Kit

Important points before starting

- If using the EZ1 ccfDNA Mini Kit for the first time, read "Important Notes", page 12.
- 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 6. Do not use damaged kit components, since their use may lead to poor kit performance or contamination of the EZ1 instrument.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See "Safety Information", page 6.
- Perform all steps of the protocol at room temperature. During the setup procedure, work quickly.

Things to do before starting

- Prepare a shaker for microcentrifuge tubes at room temperature for use in step 4.
- Ensure availability of magnetic racks for 15 ml and 2 ml tubes.
- EZ1 instruments should only be switched on after an EZ1 card is inserted. Make sure that the EZ1 Card is completely inserted, 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.
- 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.

- Resuspend Magnetic Bead Suspension by pulse-vortexing for 1 min. Do not let the suspension settle for more than 2 min. Pipet from the center of the suspension.
- Before loading reagent cartridges into the EZ1 instrument, invert them 3 times to mix the magnetic particles, and then tap to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.

Procedure

1. Mix components according to Table 2 in a 15 ml tube (not provided), and incubate for 10 min at room temperature while shaking (slow speed) end-over-end.

Table 2. Component mix (EZ1 ccfDNA Midi Kit)

Plasma (ml)	Magnetic Bead Suspension (µl)	Proteinase K (µl)	Bead Binding Buffer (µl)
4	120	220	600
5	150	275	750
6	180	330	900
7	210	385	1050
8	240	440	1200
9	270	495	1350
10	300	550	1500

2. Spin briefly (30 s at 200 x *g*) to remove any solution in cap.
3. Place the tube containing bead solution into a magnetic rack for 15 ml tubes. Let stand for at least 1 min until the solution is clear. Discard supernatant.
4. Remove the tube from the magnetic rack and add 200 µl Bead Elution Buffer to the bead pellet. Vortex to resuspend beads, and pipet up and down to mix and rinse residual beads from the tube wall. Transfer the mixture (including beads) into a Bead Elution Tube. Incubate for 5 min on a shaker for microcentrifuge tubes at room temperature and 300 rpm.

5. Place the Bead Elution Tube containing the bead solution into a magnetic rack for 2 ml tubes. Let stand for at least 1 min, until the solution is clear. Transfer supernatant (pre-eluate) to a new 1.5 ml elution tube and save for use in step 7.

   Note: Avoid transferring any magnetic beads in this step. Carryover may result in reduced ccfDNA yield.

   Note: Each sample requires two 1.5 ml elution tubes: 1 for loading the pre-eluate on the EZ1 instrument and 1 to collect ccfDNA after purification (worktable setup in step 7 will guide you).

6. Insert the EZ1 Advanced XL ccfDNA Mini/Midi Card completely into EZ1 Advanced XL Card slot of the EZ1 Advanced XL Instrument. Switch on the instrument.
7. Press **Start** to start worktable setup of the EZ1 ccfDNA Midi protocol. Open the instrument door. Follow onscreen instructions for worktable setup and data tracking. Close the door, and press **Start** to start the protocol.
8. The display shows "Protocol finished" when finished. Press **Esc**.
9. Open the instrument door. Remove elution tubes containing purified ccfDNA (in 60 µl) from the first row. Discard the sample preparation waste.* Press **Ent**. The report file is automatically transferred.

   Optional: Follow onscreen instructions for UV decontamination of table surfaces.

10. Perform regular maintenance after each run. Press **Esc** to return to the main menu.

    Note: Regular maintenance consists of cleaning the piercing unit and the worktable surfaces.

    Important: The piercing unit is sharp. The use of double gloves is recommended.

11. To run another protocol, press **Start** and follow the protocol from step 7. Otherwise, close the instrument door and switch off instrument.

* Sample waste contains guanidine salts and is not compatible with bleach.

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
Little or no nucleic acids in the eluate		
a)	Primary blood tube contains an anticoagulant other than EDTA	Anticoagulants other than EDTA may lead to accelerated DNA degradation compared to EDTA blood. Repeat the purification procedure with new samples.
b)	Extended time between blood draw and plasma preparation	Blood cells may disintegrate and release genomic DNA into the plasma, diluting the target nucleic acid.
c)	Samples frozen and thawed more than once	Repeated freezing and thawing should be avoided because this may lead to DNA degradation. Always use fresh samples or samples that have been thawed only once.
d)	Low concentration of target DNA in the samples	Samples were left standing at room temperature for too long. Repeat the purification procedure with new samples.
e)	Insufficient reagent aspirated	After inverting the reagent cartridges to resuspend the magnetic particles, make sure to tap the cartridges to deposit the reagents at the bottom of the wells.
f)	Magnetic particles not completely resuspended	Make sure to resuspend the magnetic particles thoroughly before loading the reagent cartridges into the holder.
g)	Wrong measurement method used	Due to low yields and the small size of ccfDNA fragments, not all measurement methods are reliable, see “Yield and size of nucleic acids”, page 10.

		Comments and suggestions
DNA or RNA do not perform well in downstream enzymatic reactions		
a)	Little or no DNA in the eluate	See "Little or no nucleic acids in the eluate" above for possible reasons. Increase the amount of eluate added to the reaction if possible.
b)	Inappropriate elution volume used	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly. The elution volume can be adapted proportionally.
c)	New *Taq* DNA polymerase or PCR chemistry used	If enzymes are changed, it may be necessary to readjust the amount of eluate used for PCR.
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 XL via the "PC/Printer" serial port. Check whether the serial port is set for use with a printer.
c)	Wrong Q-Card ID entered	If the wrong ID was entered instead of the Q-Card ID, the 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.

Appendix A: Recommendations for Plasma Separation and Storage

To isolate circulating, cell-free nucleic acids from blood samples, we recommend following this protocol, which includes a high *g*-force centrifugation step to remove cellular debris and reduce the amount of cellular or genomic DNA and RNA in the sample. Human serum or plasma samples can be generated using blood collection tubes such as the PAXgene Blood ccfDNA Tube; please refer to the manufacturer´s recommendation for plasma separation procedure.

Procedure

1. Place whole blood in a collection tube into a centrifuge with a swing-out rotor and appropriate buckets.
2. Centrifuge the blood samples for 10 min at 1900 x *g* (3000 rpm) with temperature set to 4°C.
3. Carefully aspirate plasma supernatant without disturbing the buffy coat layer. Approximately 4–5 ml plasma can be obtained from one 10 ml primary blood tube.

   Note: Plasma can be used for circulating nucleic acid extraction at this stage. However, the following high-speed centrifugation will remove additional cellular debris and contamination of the circulating nucleic acids by genomic DNA and RNA derived from damaged blood cells.
4. Transfer aspirated plasma into new 15 ml centrifuge tubes with conical bottoms.
5. Centrifuge the plasma samples for 10 min at 16,000 x *g* in a fixed-angle rotor with temperature set to 4°C.

   This will remove additional cellular nucleic acids attached to cell debris.
6. Using a pipette, carefully transfer the supernatant into a new tube without disturbing the pellet.

7. If plasma will be used for nucleic acid extraction on the same day, store at 2–8°C until further processing. For longer storage, keep plasma frozen at –90 to –65°C. Before using the plasma for circulating nucleic acid extraction, thaw plasma tubes at room temperature.
8. In case of cryoprecipitates, follow these 2 steps:
   8a. Centrifuge plasma sample for 5 min at 16,000 x *g* in fixed angle rotor with temperature set to 4°C.
   8b. Transfer the supernatant into a new tube, and then begin with the nucleic acid extraction protocol.

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: _ _ _ _ _ _ _ _ _ _ SN 0001

User ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 9876543210

Firmware version: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ V 1.0.0

Installation date of instrument: _ _ _ _ _ _ Jan 05, 2008

Weekly maintenance done on: _ _ _ _ _ _ Jun 15, 2008

Yearly maintenance done on: _ _ _ _ _ _ Jan 10, 2008

Date of last UV-run: _ _ _ _ _ _ _ _ _ _ _ _ _ Mar13, 2008

Start of last UV-run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 16:06

End of last UV-run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 16:26

Status UV-run: _ o.k.

Protocol name: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ Virus 2.0

_ _

Date of run: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ Jul 25, 2008

Start of run: _ 12:57

End of run: _ 13:50

Status run: _ o.k.

Error code: _

Sample input volume [ul]: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 400

Elution volume [ul]: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 150

Channel A:

Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 123456789

Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401

Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ 1181234567

Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210

Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 848373922

Note: _ 2000

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Channel B:

Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 234567890

Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401

Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ 1181234567

Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210

Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 836266738

Note: _

Channel C:

Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 345678901

Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401

Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ 1181234567

Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210

Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 883727832

Note: _ 1000

Channel D:

Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 456789012

Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401

Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ 1181234567

Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210

Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 763684837

Note: _

Channel E:

Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 567890123

Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401

Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ 1181234567

Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210

Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 4387728002

Note: _

Channel F:

Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 678901234

Reagent kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 9801401

Reagent lot number: _ _ _ _ _ _ _ _ _ _ _ 1181234567

Reagent expiry date: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 1210

Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 509389403

Note: _ 50

Ordering Information

Product	Contents	Cat. no.
EZ1 ccfDNA Mini Kit (48)	48 reagent cartridges (EZ1 ccfDNA), QIAGEN Proteinase K, Magnetic Bead Suspension, Buffers, 50 Bead Elution Tubes, 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 100 Elution Tubes (1.5 ml)	954134
EZ1 ccfDNA Midi Kit (48)	48 reagent cartridges (EZ1 ccfDNA), QIAGEN Proteinase K, Magnetic Bead Suspension, Buffers, 50 Bead Elution Tubes, 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 100 Elution Tubes (1.5 ml)	954154
EZ1 Advanced XL, System	Robotic workstation for automated purification of nucleic acids from up to 14 samples using EZ1 Kits: includes installation, training, 1-year warranty on parts and labor	9001874
EZ1 Advanced XL ccfDNA Mini/Midi Card	Preprogrammed card for EZ1 ccfDNA cleanup protocol on the EZ1 Advanced XL instrument	9025647
Related products		
QIAamp® MinElute® ccfDNA Mini Kit (50)	For 50 preps: QIAamp UCP MinElute Columns, QIAGEN Proteinase K, Magnetic Bead Suspension, Buffers, Bead Elution Tubes, and Collection Tubes (1.5 ml and 2 ml)	55204

Product	Contents	Cat. no.
QIAamp MinElute ccfDNA Midi Kit (50)	For 50 preps: QIAamp UCP MinElute Columns, QIAGEN Proteinase K, Magnetic Bead Suspension, Buffers, Bead Elution Tubes, and Collection Tubes (1.5 ml and 2 ml)	55284
PAXgene Blood ccfDNA Tubes (100)	100 blood collection tubes (10 ml). To be used in conjunction with QIAGEN QIAamp MinElute ccfDNA Kits	768115
Accessories		
Filter Tips and Holders, EZ1 (50)	50 Disposable Filter-Tips, 50 Disposable Tip Holders; additional tips and holders for use with EZ1 Kits	994900
15 ml/50 ml Tube Magnet	Magnet rack for 5 x 15 ml tubes and 3 x 50 ml tubes	36935
12-Tube Magnet	Magnet rack for 12 x 1.5/2 ml tubes	36912
AdnaMag-L	Magnetic rack for 8 tubes, 15 ml	399921
AdnaMag-S	Magnetic rack for 8 tubes, 1.5 ml	399911

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Document Revision History

Date	Changes
08/2018	Modifications to protocol to improve reader comprehensibility.
06/2020	Changed volume of Bead Elution Buffer, from 10 ml to 12 ml. Added footnote about number of preps in "Kit Contents". Added “Quality Control” statement. Updated “Safety Information”. Reworded first paragraphs in "Introduction", "Principle and workflow", "Preparing plasma from whole blood", and Appendix A for clarity. Removed documentation hardware and updated the recommended cat. no. for EZ1 Advanced XL in “Equipment and Reagents to Be Supplied by User”. Added note in step 5 of each protocol. Converted long-term storage temperature in step 7, Appendix A, to temperature range.

Notes

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