March 2025

EZ1&2™ ccfDNA Kit Handbook

For automated purification of circulating cell-free DNA (ccfDNA) from plasma or serum using the EZ1® Advanced XL or the EZ2® Connect instruments

Sample to Insight

Table of Contents

Kit Contents

EZ1&2 ccfDNA Kit **Catalog no.** **Number of preps**	**(48)** **954854** **48**
Large Volume Tube (7 mL)	4 x 24
Magnetic Bead Suspension EZ	48 x 0.075 mL
Elution Buffer EZE*	4 x 15 mL
Elution Tubes 1.5 mL	50
Reagent Cartridges, EZ1&2 ccfDNA†	48
Disposable Tip Holders	50
Disposable Filter-Tips	50
Q-Card‡	1
Quick-Start Protocol§	1

* Elution buffer EZE is supplied as a concentrate – the working solution is prepared by the EZ1 or EZ2 instrument during the automated procedure.

† Contains sodium azide as a preservative. See Safety Information.

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

§ To view this handbook's corresponding Quick-Start Protocol, go to **www.qiagen.com/HB-2891**

Shipping and Storage

EZ1&2 ccfDNA Kits are shipped at ambient temperature. Upon receipt, store all kit components dry at room temperature (15–25°C). Do not freeze the reagent cartridges. When stored properly, buffers and reagent cartridges are stable until the expiration date printed on the kit box lid.

Partially used bottles of elution buffer EZE can be stored for a maximum of 4 weeks.

Intended Use

EZ1&2 ccfDNA Kits 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 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.

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.

If liquid containing potentially infectious agents is spilt on the EZ1 Advanced XL or EZ2 Connect, please 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&2 ccfDNA Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

Circulating, cell-free DNA (ccfDNA), such as tumor-specific extracellular DNA fragments or fetal DNA in maternal blood, is present in serum or plasma usually as short fragments, <1000 bp. The concentration of cfDNA in biological fluids such as plasma or urine is usually low and varies considerably between individuals, usually from 1–100 ng/mL.

EZ1&2 ccfDNA Kits enable efficient purification of these cfDNA fragments 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. cfDNA isolation on the EZ1 Advanced XL instrument provides convenient and fully automated bead-based purification of up to 14 samples in parallel, from up to 8 mL of sample. While the cfDNA isolation on the EZ2 Connect instrument provides convenient and fully automated bead-based purification of up to 24 samples in parallel, from up to 10 mL of sample.

The eluted ccfDNA is ready for use in downstream reactions or storage at –30°C to –15°C. Purified nucleic acids are free of proteins, nucleases, and other impurities.

Principle and procedure

The EZ1&2 ccfDNA Kit combines the speed and efficiency of anion exchange-based nucleic acid purification with the convenient automated handling of magnetic particles. The purification procedure comprises 3 steps: bind, wash, and elute. It is suited for simultaneous processing of multiple samples, and provides pure nucleic acid in a short time for up to 14 samples on the EZ1 Advanced XL, or up to 24 samples on the EZ2 Connect. Run times depending on the sample volume are shown in the following table. To allow processing of these volumes of plasma or serum, the kit contains Large Volume Tubes (7 mL). To

accommodate the Large Volume Tubes, special Tip Racks have to be used. See details in section "Automation" on the next page and the "Ordering Information" on page 39.

Table 1. Sample volumes and corresponding run times

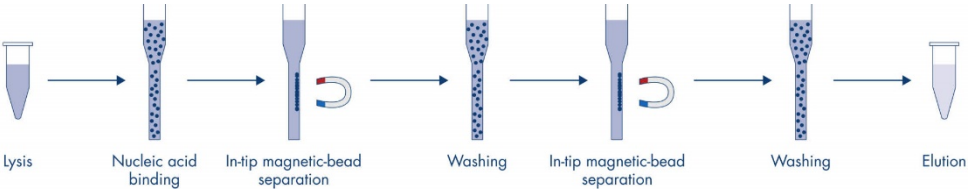
Sample volume (mL)	Run time (min)
2	39
4	49
8	73
10*	82*

* Available only for EZ2 Connect.

To isolate circulating, cell-free nucleic acids from blood samples, plasma needs to be prepared from whole blood. The obtained plasma can be stored at –30°C to –15°C in the supplied Large Volume Tubes (7 mL) until the start of the automated ccfDNA isolation. For ccfDNA isolation, the plasma samples are loaded in the Large Volume Tubes onto the instrument.

Adsorption to anion exchange magnetic particles

Proteinase K and binding buffer are automatically added to the samples provided in the Large Volume Tubes to adjust binding conditions. Samples are thoroughly mixed with magnetic particles to allow adsorption of ccfDNA to the 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 3 wash steps. Sample preparation waste is collected in tubes in positions 2, 3, and 4 of the tip rack, which holds the 7 mL large volume tubes to provide the sample and the tip holder. Remaining liquid in these positions is normal and not an indication of incomplete processing of the sample.

Elution of purified nucleic acids

Highly purified cfDNA is eluted in low salt buffer (the eluate volume can range from 35–55 µL or 60–80 µL, depending on the elution option) 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°C to –15°C if longer than 24 hours.

Automation

The EZ1&2 ccfDNA Kit can be used on the EZ1 Advanced XL instrument or the EZ2 Connect instrument. The provided protocol describes the workflow for both instruments. cfDNA binding, washing, and elution steps are completely conducted on the EZ1 Advanced XL instrument or EZ2 Connect instrument.

To accommodate the Large Volume Tubes (7 mL) that are used with the EZ1&2 ccfDNA Kits, the EZ1 Advanced XL Tip Rack - Large Volume is required, which needs to be ordered separately (cat. no. 9027008) when using the EZ1 Advanced XL instrument. In addition, the EZ1 Advanced XL ccfDNA Card (cat. no. 9026964) providing the ccfDNA protocols is also

required. When using the EZ2 Connect instrument, the EZ2 Connect Tip Rack - Large Volume is required, which needs to be ordered separately (cat. no. 9027011).

Starting material

The EZ1&2 ccfDNA Kit has been tested with serum and plasma samples.

Preparing plasma from whole blood

For recommendations for plasma preparation please refer to "Appendix A: Recommendations for Plasma Separation and Storage" on page 33. 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. The obtained plasma can be stored at –30°C to –15°C in the supplied Large Volume Tubes until the "Start" of the automated ccfDNA isolation.

Sample volumes

The EZ1&2 ccfDNA Kit has been optimized for sample volumes of 1–10 mL. The yield depends on the sample volume and the concentration of circulating nucleic acids in the sample (typically, 1–100 ng/mL in plasma). The EZ1&2 ccfDNA Kit has been designed to be flexible with serum or plasma input volumes, and provides 3 different protocols to choose from, depending on the input volume. The 2 mL protocol can be used to process sample volumes of up to 2 mL, the 4 mL protocol to process sample volumes of >2 mL to 4 mL, the 8 mL protocol to process samples >4 mL to 8 mL, and the 10 mL protocol to process sample volumes of >8 to 10 mL.

The provided large volume tubes can be used for storage of the generated plasma at –30°C to –15°C until used on the instrument.

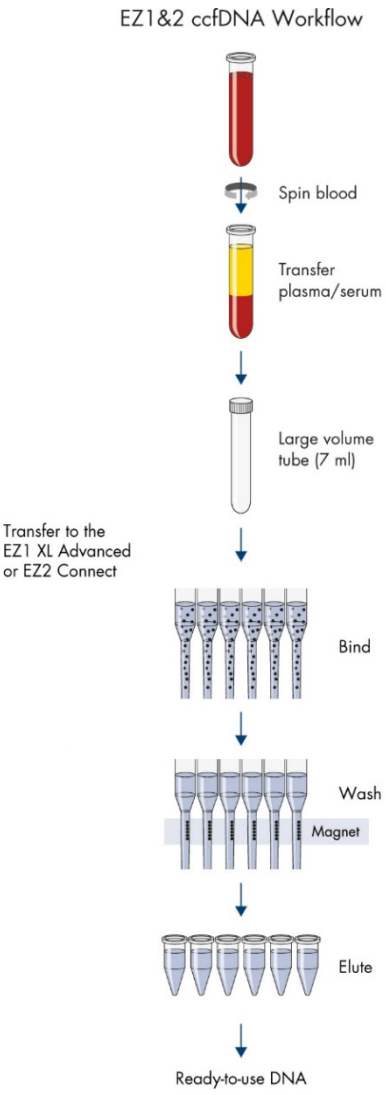
Sample numbers

The kit provides enough material to run 48 samples in total. Using the EZ1 Advanced XL instrument up to 14 samples can be run in parallel and up to 24 when using the EZ2 Connect.

Yield and size of nucleic acids

Yields of cell-free circulating nucleic acids isolated from biological samples are normally well below 1 µg and are therefore too low 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 using a multi-copy target gene with known and stable copy number per genome (e.g., GAPDH or β-actin) is the most accurate method for determining the concentration of small amounts of cfDNA and recommended for determination of yields.

Size distribution of circulating nucleic acids purified using this procedure can be checked by analysis on QIAxcel® Connect, or a similar device.


EZ1&2 ccfDNA Workflow
Spin blood
Transfer plasma/serum
Large volume tube (7 ml)
Transfer to the EZ1 XL Advanced or EZ2 Connect
Bind
Wash
Magnet
Elute
Ready-to-use DNA

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&2 ccfDNA Kit, the following supplies are required.

- Vortex shaker for microcentrifuge tubes
- Pipettes (adjustable)
- Sterile pipette tips (pipette tips with aerosol barriers are recommended to help prevent cross-contamination)
- For non-stabilized urine samples: Buffer ATL (cat. no. 19076 or 939011)*
    - EZ1 Advanced XL:
        - EZ1 Advanced XL instrument (cat. no. 9001874)
        - EZ1 Advanced XL ccfDNA Card (cat. no. 9026964)
        - EZ1 Advanced XL Tip Rack – Large Volume (cat. no. 9027008)
    - EZ2 Connect:
        - EZ2 Connect instrument (cat. no. 9003210)
        - EZ2 Connect Tip Rack – Large Volume (cat. no. 9027011)

*Product is not part of the EZ1&2 ccfDNA Kit and it has to be ordered separately (see section Ordering Information - Relative Products)

Important Notes

- This handbook contains instructions on using the EZ1&2 ccfDNA Kit on the EZ1 Advanced XL and the EZ2 Connect instruments. The next section will describe the respective instruments and their specific requirements for this kit (e.g., use of a special rack, starting on page 15).
- The protocol on page 22 will include information on sample handling on the EZ1 Advanced XL (starting on page 26) and the EZ2 Connect (starting on page 25) instrument.
- 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.

Reagent cartridges

Reagents for purification of nucleic acids from a single sample are contained in a single reagent cartridge (Figure 1, next page). Each well of the cartridge contains a particular reagent, such as binding buffer or wash buffer.

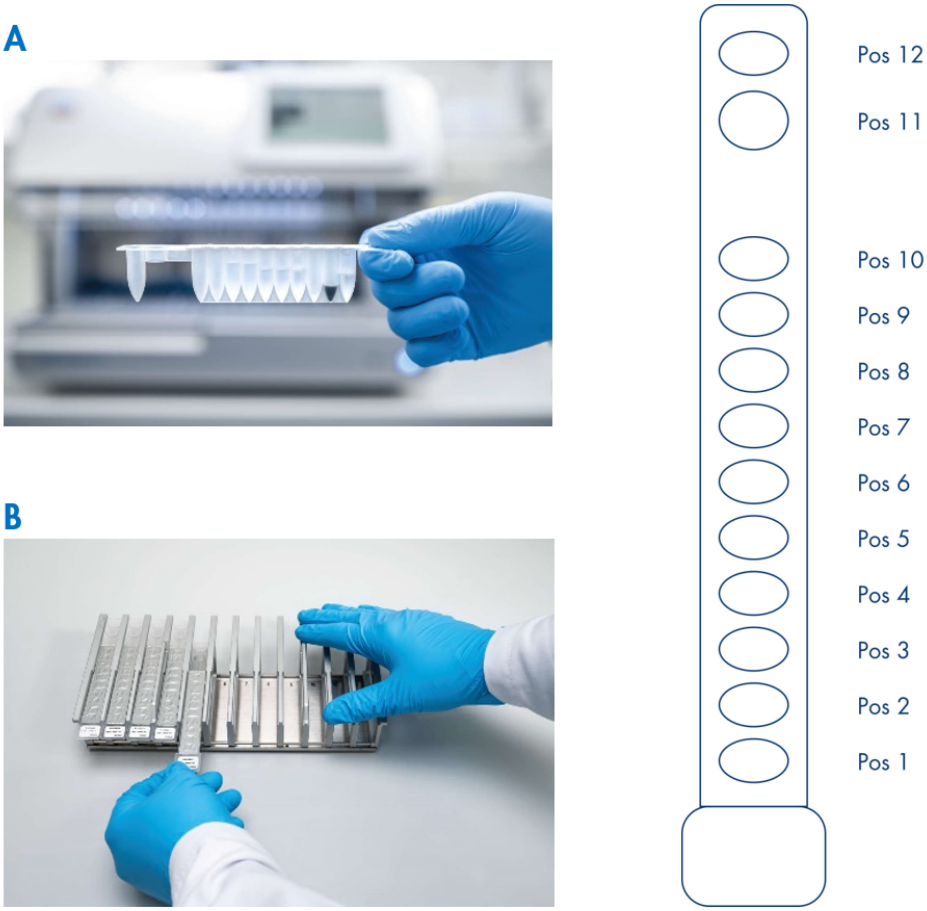


Figure 1. Ease of worktable setup using reagent cartridges. [A] A sealed, prefilled reagent cartridge (example). **[B]** Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded.

Working with the EZ2 Connect instrument

Worktable

The worktable of the EZ2 Connect instrument is where the user loads samples and the components of the EZ1&2 ccfDNA Kit (cat. no. 954854).

Details on worktable setup are provided in the protocol in this handbook and are also displayed on the touch screen when the user starts the worktable setup. The display also shows protocol status during the automated purification procedure.

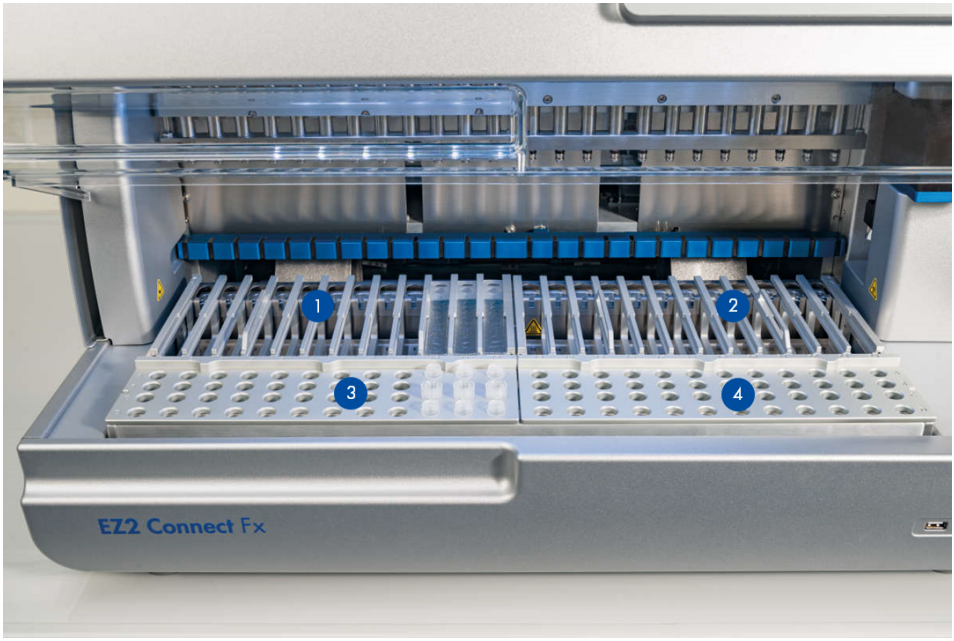


Figure 2. EZ2 Connect worktable. **[1]** EZ2 Connect Cartridge Rack, left. **[2]** EZ2 Connect Cartridge Rack, right. **[3]** EZ2 Connect Tip Rack, left. **[4]** EZ2 Connect Tip Rack, right.

Operation of the EZ2 Connect

The EZ2 Connect provides various features to support the sample preparation workflow. These include functions for remote access via QIAsphere, data input via bar code reading, data storage and transfer, report generation, and guided instrument maintenance. For more information about these features, please refer to the EZ2 Connect and EZ2 Connect Fx User Manual.

EZ2 Connect Cartridge Rack

The EZ2 Connect Cartridge Rack consists of two separate parts. The left cartridge rack is used for cartridges in positions 1 to 12. The right cartridge rack is used for cartridges in positions 13 to 24. On the worktable, the cartridge rack is located behind the tip rack (Figure 2). It holds up to 24 reagent cartridges.

EZ2 Connect Tip Rack

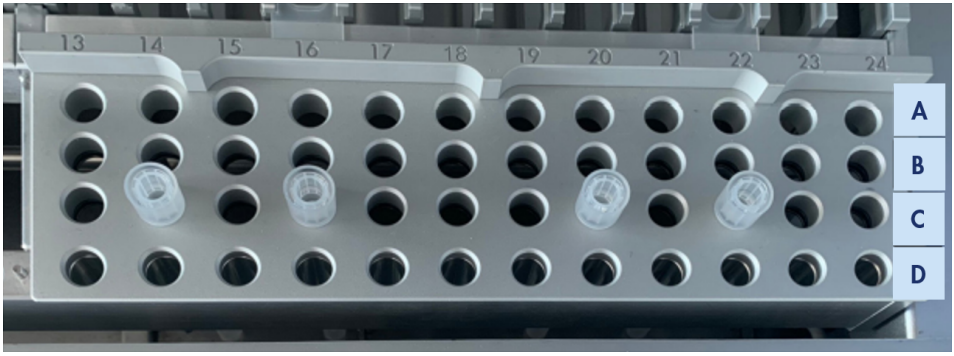


Figure 3. EZ2 Connect Tip Rack.

The EZ2 Connect Tip Rack consists of 2 separate parts. The left part of the tip rack is used for labware in positions 1 to 12. The right part of the tip rack is used for labware in positions 13 to 24. The EZ2 Connect Tip Racks are located at the front of the worktable (Figure 2). Each

consists of 4 rows and 12 columns. Individual positions in the EZ2 Connect Tip Rack are marked by engravings. During run setup, the user interface gives instructions to load specific positions of the EZ2 Connect Tip Rack with filter tips in tip holders, sample tubes, or elution tubes.

Important: To accommodate the Large Volume Tubes (7 mL) that are used with the EZ1&2 ccfDNA Kits, the EZ2 Connect Tip Rack - Large Volume is required, which needs to be ordered separately (cat. no. 9027011). Remove the standard racks from the instrument and replace them with the EZ2 Connect Tip Rack - Large Volume.

Working with the EZ1 Advanced XL instrument

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 4 and Figure 5). The availability of various protocols increases the flexibility of EZ1 instruments.

Figure 4. Ease of protocol setup using EZ1 Advanced XL Cards. Inserting an EZ1 Card, which contains a protocol, into the EZ1 Advanced XL instrument.

Figure 5. EZ1 Card completely inserted into EZ1 Card slot.

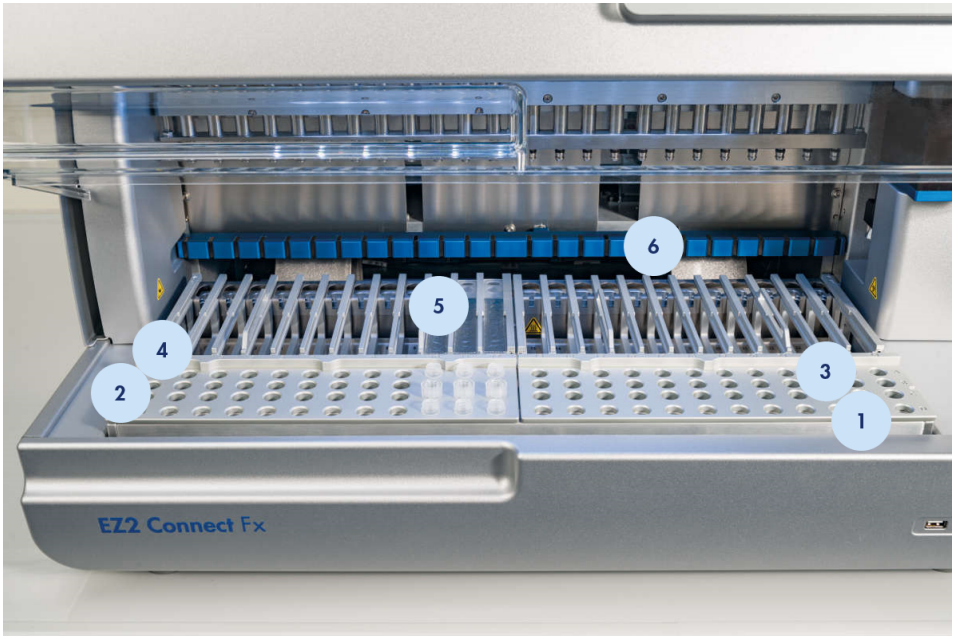
The instrument should only be switched on after an EZ1 Card is inserted. Make sure that the EZ1 Card is completely inserted (Figure 5). Otherwise, essential instrument data could be lost,

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.

Worktable

The worktable of the EZ1 Advanced XL instrument is where the user loads samples and the components of the EZ1&2 ccfDNA Kit.

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



[1] First row: Elution tubes (ET) (1.5 mL) are loaded here. **[2]** Second row: Large volume tubes containing 2 or 4 mL (or 5 mL for 10 mL protocol) of sample are loaded here. After the run is completed, the tubes contain liquid waste. **[3]** Third row: for 2 and 4 mL protocols, new (empty) large volume tubes are loaded here. For the 8 mL or 10 mL protocols, large volume tubes containing 4 mL or 5 mL of sample are loaded here, respectively. After the run is completed, the tubes contain liquid waste. **[4]** Fourth row: Disposable tip holders (DTH) containing disposable filter tips (DFT) are loaded here. **[5]** Reagent cartridges (RCB) loaded into the cartridge rack. **[6]** The heating block positions contain magnetic bead suspension EZ and elution buffer EZE placed by the user before the start of the EZ1&2 ccfDNA Kit protocol.

EZ1 Advanced XL Tip Rack

Important: To accommodate the Large Volume Tubes (7 mL) that are used with the EZ1&2 ccfDNA Kits, the EZ1 Advanced XL Tip Rack - Large Volume is required, which needs to be ordered separately (cat. no. 9027008) when using the EZ1 Advanced XL instrument. Remove the standard racks from the instrument and replace them with the EZ2 Connect Tip Rack - Large Volume.

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&2 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" on page 35. 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 Enter 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, **www.qiagen.com/HB-0176** to access the *EZ1 Advanced XL User Manual*.

Protocol: Purification of ccfDNA from up to 10 mL Serum or Plasma Using the EZ1&2 ccfDNA Kit

Important points before starting

- If using the EZ1&2 ccfDNA Kit for the first time, read "Important Notes" on page 13.
- 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" on page 5. Do not use damaged kit components, because their use may lead to poor kit performance or contamination of the instrument.
- Make sure that the EZ1 Advanced XL Tip Rack - Large Volume or EZ2 Connect Tip Rack - Large Volume is used instead of the regular tip rack.
- The 10 mL protocol is available only for the EZ2 Connect.

Things to do before starting

- Plasma samples should be at room temperature before use. If plasma/serum was stored frozen, thaw the samples by incubating at 37°C in a water bath.
- If the sample volume is less than 2 mL, 4 mL, 8 mL, or 10 mL, adjust the volume with PBS to 2 mL, 4 mL, 8 mL, or 10 mL.
- It is very important to fill the large volume tubes with the volume specified (2 mL, 4 mL, or 5 mL) and not more or less as this can lead to foaming or sample spillage resulting in poor ccfDNA quality and yield.
- 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.

Procedure

1. Load reagent cartridges into the cartridge rack.
2. Vortex the Magnetic Bead Suspension EZ briefly in an upright position.
3. Turn the tube with the cap facing downwards and vortex again to ensure a homogeneous bead suspension.
4. Briefly centrifuge to collect all the liquid at the bottom of the tube. Remove the cap from the tube with the bead solution and place in position 11 of the EZ1&2 ccfDNA cartridge (see Figure 6).

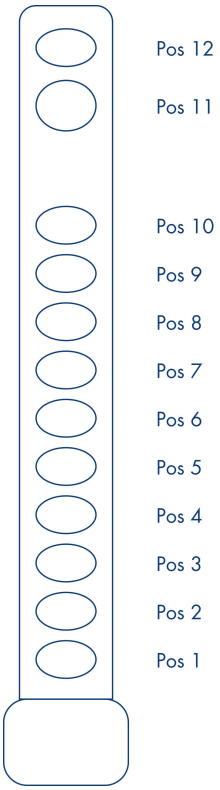


Figure 6. EZ1&2 ccfDNA cartridge.

5. Transfer 900 µL elution buffer EZE into position 12 of the EZ1&2 ccfDNA cartridge (see Figure 6).
6. Remove caps of all tubes and prepare the large volume tip rack as follows (see Figure 7):
    - Position 4/A: Tip holder with Filter Tip (provided)
    - Position 3/B: new large volume tube (7 mL) (provided)

- Position 2/C: new large volume tube (7 mL) (provided)
- Position 1/D: new 1.5 mL elution tube (provided)

Figure 7. Tip Rack - Large Volume.

7. For 2 mL/4 mL protocol: Transfer exactly 2 mL/4 mL sample into the large volume tube in position 2/C. Place an empty large volume tube in position 3/B.

   For 8 mL protocol: Split your sample and transfer exactly 4 mL into each of the 7 mL tubes in positions 2/C and 3/B.

   For 10 mL protocol: Split your sample and transfer exactly 5 mL into each of the 7 mL tubes in positions 2/C and 3/B.

Procedure on the EZ2® Connect

1. Turn on the EZ2 Connect instrument.
2. Tap **DNA** on the Applications panel and select the **EZ1&2 ccfDNA Kit** and press **Next**. Follow onscreen instructions for selection of protocol, parameter definition, sample position selection, sample IDs, and worktable setup.

3. Open the instrument door. Load the cartridge rack into the instrument.
4. Place the EZ2 Connect Tip Rack - Large Volume into the instrument.
5. Close the instrument door; press **Start** to initiate the EZ1&2 ccfDNA protocol.
6. The display will show "Protocol finished" when the run is completed. Select **Finish**.

   Open the instrument hood. Remove the elution tubes containing the purified ccfDNA position 1/D of the tip rack. Discard the sample preparation waste (in tubes in positions 2/C and 3/B, and in case of the 8 mL and 10 mL protocols, in position 4/A) (see Figure 7 on the previous page).

   Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.
7. Perform regular maintenance after each run. Press **Finish** to return to the Home Screen.

Procedure on the EZ1 Advanced XL

1. For use on EZ1 Advanced XL instrument, insert the EZ1 Advanced XL ccfDNA Card completely into the card slot and turn on the instrument. Please make sure the instrument is turned off prior to inserting the card.
2. Press **Start** to initiate the EZ1&2 ccfDNA protocol. Follow onscreen instructions for selection of protocol, data tracking, and worktable setup. Close the instrument door; press **Start** to start the protocol.
3. Open instrument door. Load the cartridge rack into the instrument.
4. Place the EZ1 Advanced XL Tip Rack - Large Volume into the instrument.
5. The display shows "Protocol finished" when finished. Press the Esc key.
6. Open the instrument door. Remove elution tubes containing purified ccfDNA (in 75 µL) from position 1 of the tip rack (see Figure 7 on the previous page). Discard the sample preparation waste (in tubes in positions 2 and 3 and, in case of the 8 mL protocol, in

position 4) (see Figure 7). Press the Enter key. The report file is automatically transferred.

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

7. Perform regular maintenance after each run. Press the Esc key 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.

8. To run another protocol, press **Start** and follow the protocol from step 1. Otherwise, close the instrument door and switch off the instrument.

Protocol: Purification of ccfDNA from up to 10 mL Urine Using the EZ1&2 ccfDNA Kit

Stabilized human urine

Important: Stabilize urine samples immediately due to the rapid degradation of circulating cell-free DNA after urine collection.

- Collect urine samples and stabilize using a commercial solution.

  Note: Using additives such as EDTA alone does not sufficiently stabilize the cfDNA in the urine.

- Centrifuge urine samples at low speed (1900 x *g*) for 10 min at room temperature (15–25°C) to remove cells prior to extraction of circulating cell-free DNA.

Non-stabilized human urine

Important: Stability and integrity of circulating cell-free DNA is limited in non-stabilized urine; work quickly during ccfDNA extraction.

Note: Buffer ATL is not part of the EZ1&2 ccfDNA Kit and it has to be ordered separately (see section Ordering Information - Relative Products)

We do not provide a ready-to-use stabilizing solution but recommend the following pre-treatment:

- Before starting a protocol that requires Buffer ATL, check whether precipitate has formed in Buffer ATL. If necessary, dissolve by heating at 70°C with gentle agitation in a water bath. Aspirate bubbles from the surface of Buffer ATL.

- It is recommended to centrifuge urine samples immediately after collection at low speed (1900 x *g*) for 10 min at room temperature (15–25°C) to remove cells. Non-stabilized urine samples require sample pretreatment.

Important: Equilibrate samples to room temperature (15–25°C) before starting pretreatment.

Important: Centrifugation and pretreatment should be performed within 4 h of urine sample collection.

1. Mix urine and Buffer ATL in a ratio of 10:1 (e.g., 1800 µL , 3600 µL, or 4500 µL urine with 200 µL, 400 µL, or 500 µL Buffer ATL, respectively). Incubate the samples at room temperature (15–25°C) for 1 hour.
2. Centrifuge samples at 1900 x *g* for 10 min at room temperature (15–25°C).

Procedure for processing human urine samples

1. If precipitates are visible in the stabilized urine (e.g., after thawing), warm the samples to 25°C in a water bath to dissolve them.
    a. Stabilized urine as sample input can be used directly with the EZ1&2 ccfDNA Kit the same as serum or plasma samples.
    b. Non-stabilized urine samples should be pretreated as described.
2. Transfer the supernatant to the large volume tube according to the "Procedure on the EZ2® Connect" section of "Protocol: Purification of ccfDNA from up to 10 mL Serum or Plasma Using the EZ1&2 ccfDNA Kit" on page 25.

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** (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Little or no nucleic acids in the eluate	
a) 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.
b) 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.
c) Magnetic particles not completely resuspended	Make sure to resuspend the magnetic particles thoroughly and no beads remain in the caps when they are removed, before loading the tubes into position 11 of the reagent cartridges.
d) Foaming during purification	Make sure that the large volume tubes are filled with 2 mL (2 mL protocol), 4 mL (4 mL or 8 mL protocol for both tubes) or 5 mL (10 mL protocol for both tubes) of sample and not less, as this can cause foaming, which may drastically reduce ccfDNA yield and purity.
e) 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" on page 10.
f) Elution buffer EZE expired	Exchange with surrounding air may lead to reduced stability of elution buffer EZE leading to reduced efficiency of ccfDNA extraction. Close partially used bottles carefully after use, and do not use remaining elution buffer EZE more than 4 weeks after opening the bottle. The volume of elution buffer EZE provided is sufficient to allow discarding the last 2–3 mL of each bottle.
Co-purification of cellular DNA	
a) Extended time between blood draw and plasma preparation	Blood cells may disintegrate and release genomic DNA into the plasma, diluting the target nucleic acid. If blood cannot be processed to plasma immediately, use of dedicated collection tubes with ccfDNA stabilization (e.g., PAXgene Blood ccfDNA Tubes) is recommended.

	Comments and suggestions
DNA does not perform well in downstream enzymatic reactions	
a) Little or no DNA in the eluate	See "Little or no nucleic acids in the eluate" on the previous page above for possible reasons. Increase the amount of eluate added to the reaction if possible.
b) 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 Advanced XL or EZ2 Connect 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.

Reference

1. Sambrook, J., and Russell, D.W. (2001). Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

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* 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 15 min at 3000 x *g* in a fixed-angle rotor with temperature set to 4°C.

   This will remove additional cellular nucleic acids attached to cell debris.

Note: For certain blood collection tubes, specific centrifugation conditions may be recommended by the manufacturer. For example, for the PAXgene Blood ccfDNA tubes, 1900 x *g* at room temperature for 15 min is recommended for the second centrifugation.

The second centrifugation may also be performed at 10,000 or up to 16,000 x *g*. This will remove some of the larger extracellular vesicles (EVs) and cell fragments, which may also contain cell-free DNA.

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°C to –65°C. Before using the plasma for circulating nucleic acid extraction, thaw plasma tubes at room temperature or at 37°C in a water bath.
8. In case of cryoprecipitates, follow these 2 steps:
    a. Centrifuge the plasma sample using the same conditions as in step 5.
    b. 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 [µL]: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 400

Elution volume [µL]: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 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&2 ccfDNA Kit (48)	48 reagent cartridges (EZ1&2 ccfDNA), 96 Large Volume Tubes 7 mL, Elution Buffer EZE, Magnetic Bead Suspension EZ, Buffers, 50 Elution Tubes, 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 100 Elution Tubes (1.5 mL)	954854
EZ1 Advanced XL Tip Rack - Large Volume	Metal rack that accommodates tip holders containing filter-tips, elution tubes, and large volume tubes on the worktable.	9027008
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 Card	Preprogrammed card for EZ1 ccfDNA protocol on the EZ1 Advanced XL instrument	9026964
EZ2 Connect instrument	Benchtop instrument for automated isolation of nucleic acids from up to 24 samples in parallel, using sealed prefilled cartridges; includes 1-year warranty on parts and labor.	9003210
EZ2 Connect Tip Rack - Large Volume	Metal rack that accommodates tip holders containing filter-tips, elution tubes, and large volume tubes on the worktable.	9027011
Related Products		
PAXgene Blood ccfDNA Tubes (100), RUO*	100 blood collection tubes (10 mL)	768115
Buffer ATL	Lysis buffer for use in nucleic acid purification. Available in 2 sizes: 200 mL and 4 x 50 mL	19076 939011
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

* Not available in all countries

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Document Revision History

Date	Changes
08/2023	Added “Protocol: Purification of ccfDNA from up to 8 mL Urine Using the EZ1&2 ccfDNA Kit”.
03/2025	Added protocol for 10 mL sample volume. Updated the protocol for urine samples.

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