August 2022

EZ1&2 DNA Tissue Handbook

For automated purification of DNA from tissue and other samples using EZ1 instruments

Sample to Insight

Contents

Kit Contents

EZ1&2 DNA Tissue Kit*	**(48)**
Catalog no.	**953034**
Number of preps	**48**
Reagent Cartridge, Tissue (1023869)	48
Disposable Tip Holders	50
Disposable Filter-Tips	50
Sample Tubes (2 ml)	50
Elution Tubes (1.5 ml)	50
Buffer G2	1 x 13 ml
Proteinase K	2 x 250 µl
Buffer AVE	2 ml
Q-Card†	1
Quick-Start Protocol	1

* For details about the EZ1 Cards to be used with this kit, see Table 2, page 16, 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&2 DNA Tissue Kit is shipped at ambient temperature. All buffers and reagents can be stored at room temperature (15–25°C). Do not freeze the reagent cartridges. When stored properly, the reagent cartridges are stable until the expiration date on the Q-Card.

The ready-to-use proteinase K solution is stable for up to one year after delivery when stored at room temperature. To prolong the lifetime of proteinase K, storage at 2–8°C is recommended.

Intended Use

The EZ1&2 DNA Tissue Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

The EZ1&2 DNA Tissue Kit is intended to be used with EZ1 or EZ2 Connect instruments from QIAGEN®.

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, clean the affected area first with laboratory detergent and water, and then with disinfectants and detergents compatible with metallic surfaces as listed in the *EZ1 Advanced User Manual*.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of EZ1&2 DNA Tissue Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The EZ1&2 DNA Tissue Kit is for purification of genomic DNA from tissue and other samples. This handbook describes processing of this kit with EZ1 instruments. For usage of EZ1&2 DNA Tissue Kit with EZ2 instruments, please refer to the handbook (**www.qiagen.com/HB-2974-001**) and quick-start protocol (**www.qiagen.com/HB-2973-001**).

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 instrument performs all steps of the sample preparation procedure, and the procedure can be scaled up or down, allowing purification from varying amounts of starting material. Up to 14 samples are processed in a single run.

Principle and procedure

Magnetic-particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (see flowchart, page 20). 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.

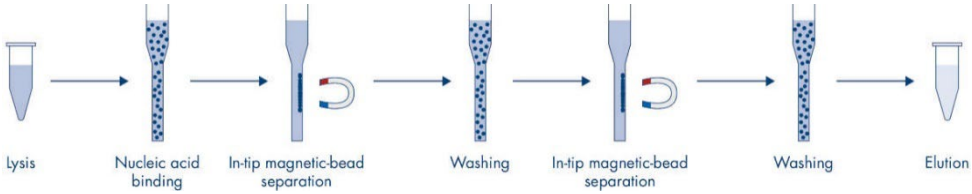

Figure 1. EZ1&2 DNA Tissue Kit workflow.

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

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

For BioRobot® EZ1 users

- BioRobot EZ1 and disposables
- EZ1 Card (see Table 2, page 16)

For EZ1 Advanced users

- EZ1 Advanced
- EZ1 Advanced Card (see Table 2, page 16)

For EZ1 Advanced XL users

- EZ1 Advanced XL
- EZ1 Advanced XL Card (see Table 2, page 16)

For EZ1 Advanced and EZ1 Advanced XL users

For documentation purposes, one of the following is required:

- EZ1 Advanced Communicator software (supplied with the EZ1 Advanced and the EZ1 Advanced XL), PC (can be connected with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments), PC and monitor.
- EZ1 Advanced Communicator Software (supplied with the EZ1 Advanced and the EZ1 Advanced XL) and your own PC and monitor (connection with up to 4 EZ1 Advanced and EZ1 Advanced XL instruments not recommended)
- Printer and accessory package for printer

For Tissue Protocol and Paraffin-Embedded Tissue Protocol

- 1.5 ml screw-capped tube

For Buccal Cells Protocol

- Swabs (for swab types tested with the protocol, see “Important points before starting”, page 26).

For Dried Blood Protocol

- Filter paper (e.g., QIAcard® FTA® Spots, see “Ordering Information”, page 60)
- Manual paper punch, 3 mm (e.g., Harris UNI-CORE 3.00 mm Punch Kit (4), cat. no. 159331, or equivalent punch with cutting mat)

For Bacterial DNA from Primary Samples or Bacterial Culture Protocols

- **Optional**: Dithiothreitol (DTT); see “Viscous or mucous samples” on page 44
- **Optional**: Phosphate-buffered saline (PBS)
- **Optional**: Carrier RNA (cat. no. 19073); see “Addition of internal control when purifying bacterial DNA from primary samples”, page 12
- **Optional**: Lysozyme (50 mg/ml in water) and/or lysostaphin (5 mg/ml in water)
- **Optional**: Glass beads, ≤106 µm (Sigma, cat. no. G8893)

Important Notes

Starting material

The amounts of starting material for use in EZ1&2 DNA Tissue procedures are shown in Table 1.

Table 1. Amounts of starting material for EZ1&2 DNA Tissue procedures

Sample type	Amount of starting material	Elution volume
Tissue	10–40 mg (see Table 3, page 21)	50 µl, 100 µl, or 200 µl
Buccal cells	1 swab or brush (approx. 200 µl volume after proteinase K digestion)	100 µl
Cultured cells	2 x 106 cells resuspended in 200 µl Buffer G2	50 µl, 100 µl, or 200 µl
Dried blood	4 discs*	50 µl, 100 µl, or 200 µl
Paraffin-embedded tissue	One to five 10 µm-thick sections (approx. 200 µl volume after proteinase K digestion)	50 µl, 100 µl, or 200 µl
Bacteria in primary samples	200 µl	50 µl, 100 µl, or 200 µl
Bacterial culture or colonies	200 µl culture or resuspended colonies, fresh or frozen	50 µl, 100 µl, or 200 µl

* A 3 mm diameter disc punched out from filter paper stained with dried blood contains white blood cells from approximately 5 µl whole blood; we recommend using 4 punched-out discs as starting material.

Buccal cells

Collection of buccal (or epithelial) cells from the inside of the cheek is a simple, inexpensive way to collect material for DNA purification. Buccal cell samples may be processed on the same day as collection or stored for future processing. While storage at –30 to –15°C is recommended, DNA of suitable quality for single-copy gene amplification has been documented from swabs stored at room temperature for 24 months.

To collect a sample, scrape the swab or brush against the inside of each cheek 6 times. Allow the swab or brush to air-dry for at least 2 h after collection. Ensure that the person providing the sample has not consumed any food or drink for 30 minutes prior to sample collection.

Paraffin-embedded tissue

For most tissue types, a sample size of one to five 10 µm-thick paraffin sections is recommended; however, up to 10 or more very small sections such as needle biopsies may be used.

The wax block should preferably be trimmed prior to sectioning in order to reduce the amount of wax in the sample material. It is recommended to trim the paraffin wax block if more than 6 sections are to be used in the DNA purification procedure. This is especially important for needle biopsy samples.

Depending on the preparation and age of the sample, some degree of DNA degradation prior to DNA purification is to be expected in paraffin-embedded tissue sections.

Bacterial culture

Both fresh and frozen bacterial cultures as well as plate-grown colonies may be used as starting material. The recommended amount of starting material to use per purification is either ≤200 µl overnight culture or several plate-grown colonies resuspended in 200 µl Buffer G2 (supplied). For some bacterial species, up to 1 ml culture (resuspended in 200 µl Buffer G2) can be used. The starting and elution volumes to use are given in Table 1, page 11.

Addition of internal control when purifying bacterial DNA from primary samples

Use of the EZ1&2 DNA Bacteria procedure in combination with commercially available amplification systems may require the introduction of an internal control into the purification procedure. Internal control DNA should be added directly to the sample.

For optimal purification, internal control DNA molecules should be at least 200 nucleotides long, as smaller molecules are not recovered efficiently. Refer to the manufacturer's instructions in order to determine the optimal concentration. Using a concentration other than that recommended may reduce amplification efficiency.

If the internal control DNA is purified in the absence of primary sample material (e.g., as a reference), an additional 2 µg carrier RNA should be added. Dilute the carrier RNA to a final concentration of 10 µg/ml using Buffer AVL (cat. no. 19073; supplied together with carrier RNA). The total amount of Buffer AVL, carrier RNA, and internal-control DNA should not exceed 200 µl.

Precipitate in reagent cartridge

The buffer in well 1 of the reagent cartridge (the well that is nearest to the front of the EZ1 instrument when the reagent cartridge is loaded) may form a precipitate upon storage. If necessary, redissolve by mild agitation at 37°C and then place at room temperature (15–25°C).

Lysis with proteinase K

The EZ1&2 DNA Tissue Kit contains proteinase K, which is the enzyme of choice for lysis buffers used in the Tissue Protocol. Proteinase K is isolated from the saprophytic fungus *Tritirachium album* and is particularly suitable for short digestion times. It possesses a high specific activity, which remains stable over a wide range of temperatures and pH values, with substantially increased activity at higher temperatures. The activity of the proteinase K solution is 600 mAU/ml solution (or 40 mAU/mg protein). This activity provides optimal results in EZ1&2 DNA Tissue Protocols.

Quantification of DNA

Carryover of magnetic particles may affect the absorbance reading at 260 nm (A260) of the purified DNA but should not affect downstream applications. The measured absorbance at 320 nm (A320) should be subtracted from all absorbance readings. See "Quantification of DNA", page 55, for more information.

Note: Make sure to calibrate the spectrophotometer with a suitable dilution buffer.

Working with EZ1 instruments

The main features of EZ1 instruments include:

- Purification of high-quality nucleic acids from 1–6 or 1–14 samples per run
- Small footprint to save laboratory space
- Preprogrammed EZ1 Cards containing ready-to-use protocols for nucleic acid purification
- Prefilled, sealed reagent cartridges for easy, safe, and fast setup of EZ1 instruments
- Complete automation of nucleic acid purification, from opening of reagent cartridges to elution of nucleic acids, with no manual centrifugation steps

Additional features of the EZ1 Advanced and the EZ1 Advanced XL include:

- Bar code reading and sample tracking
- Kit data tracking with the Q-Card provided in the kit
- UV lamp to help eliminate sample carryover from run-to-run and to allow pathogen decontamination on the worktable surfaces

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

EZ1 Cards

Protocols for nucleic acid purification are stored on preprogrammed EZ1 Cards (integrated circuit cards). The user simply inserts an EZ1 Card into the BioRobot EZ1, or an EZ1 Advanced Card into the EZ1 Advanced, or an EZ1 Advanced XL Card into the EZ1 Advanced XL, and the instrument is then ready to run a protocol (Figure 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, containing protocol, into an EZ1 instrument. The instrument should only be switched on after an EZ1 Card is inserted. EZ1 Cards should not be exchanged while the instrument is switched on.

EZ1 instruments should only be switched on after an EZ1 Card is inserted. Make sure that the EZ1 Card is completely inserted (Figure 3), otherwise essential instrument data could be lost, leading to a memory error. EZ1 Cards should not be exchanged while the instrument is switched on.

Figure 3. Complete insertion of EZ1 Card. The EZ1 Card must be completely inserted before the EZ1 instrument is switched on.

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

Table 2. Purification of DNA from various sample types using the EZ1&2 DNA Tissue Kit

Sample type	EZ1 Card required
Tissue	EZ1 Advanced XL DNA Tissue Card,* EZ1 Advanced DNA Tissue Card,† or EZ1 DNA Tissue Card‡
Buccal cells	EZ1 Advanced XL DNA Buccal Swab Card,* EZ1 Advanced DNA Buccal Swab Card,† or EZ1 DNA Buccal Swab Card‡
Paraffin section	EZ1 Advanced XL DNA Paraffin Section Card,* EZ1 Advanced DNA Paraffin Section Card,† or EZ1 DNA Paraffin Section Card‡
Dried blood	EZ1 Advanced XL DNA Dried Blood Card,* EZ1 Advanced DNA Dried Blood Card,† or EZ1 DNA Dried Blood Card‡
Cultured cells	EZ1 Advanced XL DNA Tissue Card,* EZ1 Advanced DNA Tissue Card,† or EZ1 DNA Tissue Card‡
Bacteria in primary samples	EZ1 Advanced XL DNA Bacteria Card,* EZ1 Advanced DNA Bacteria Card,† or EZ1 DNA Bacteria Card‡
Bacterial culture samples	EZ1 Advanced XL DNA Bacteria Card,* EZ1 Advanced DNA Bacteria Card,† or EZ1 DNA Bacteria Card‡

* A 3 mm diameter disc punched out from filter paper stained with dried blood contains white blood cells from approximately 5 µl whole blood; we recommend using 4 punched-out discs as starting material.

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

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

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. Since each well contains only the required amount of reagent, generation of waste due to leftover reagent at the end of the purification procedure is avoided.

Figure 4. Ease of worktable 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.

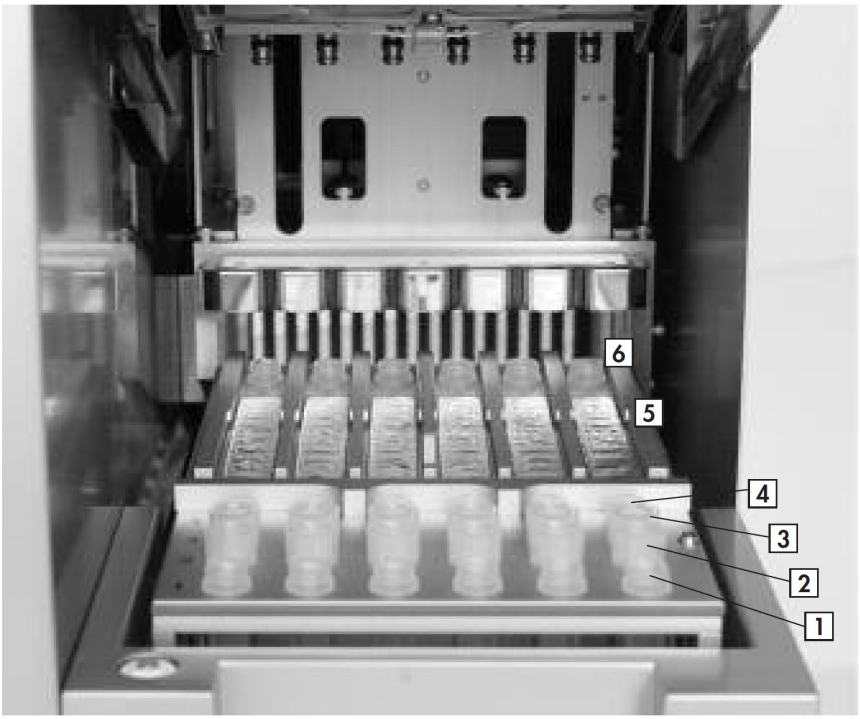

Figure 5. Typical EZ1 worktable.

1. First row: Elution tubes (1.5 ml) are loaded here.
2. Second row: Tip holders containing filter-tips are loaded here.
3. Third row: Tip holders containing filter-tips are loaded here. (In some protocols, this row is empty or loaded with 2 ml Sarstedt tubes.)
4. Fourth row: Sample tubes (2 ml) are loaded here.
5. Reagent cartridges are loaded into the cartridge rack.
6. Heating block with 2 ml tubes in the reagent cartridges for lysis.

Data tracking with the EZ1 Advanced and EZ1 Advanced XL

The EZ1 Advanced and EZ1 Advanced XL enable complete tracking of a variety of data for increased process control and reliability. The EZ1 Kit lot number and expiration 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 57). In report files, the 6 pipetting channels of the EZ1 Advanced are named, from left to right, channels A to F, or the 14 pipetting channels of the EZ1 Advanced XL are named, from left to right, channels 1–14.

When scanning a user ID or Q-Card bar code with the bar code reader, a beep confirms data input. After the information is displayed for 2 seconds, it is automatically stored, and the next display message is shown. When scanning sample ID, assay kit ID, or notes, a beep confirms data input, the information is displayed, and a message prompts you to enter the next item of information. After scanning sample ID, assay kit ID, and notes, press **ENT** once to confirm that the information entered is correct. If, for example, a wrong bar code was scanned for one of the samples, press **ESC** and then rescan all sample bar codes according to the onscreen instructions. For user ID and notes, you can enter the numbers using the keypad, or you can easily generate your own bar codes to encode these numbers.

For details about data tracking and using EZ1 Advanced Communicator software, see the *EZ1 Advanced User Manual* or the *EZ1 Advanced XL User Manual*.

Workflow of EZ1 operation

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*

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.

* EZ1 Advanced and EZ1 Advanced XL only.

Table 3. DNA yields obtained from different sample types using EZ1&2 DNA procedures

Sample type	Sample amount	DNA yield
Skeletal muscle	200 µl (40 mg tissue digested)	Up to 9 µg
Heart	200 µl (20 mg tissue digested)	Up to 12 µg
Spleen	200 µl (10 mg tissue digested)	Up to 28 µg
Lung	200 µl (10 mg tissue digested)	Up to 17 µg
Kidney	200 µl (10 mg tissue digested)	Up to 18 µg
Buccal cells	1 swab	1–4.5 µg
Cultured HL-60 cells	2 x 10^6	6–7.5 µg
Dried blood	4 x 3 mm diameter discs	0.2–0.5 µg
Bacterial culture:		
Escherichia coli	200 µl	6.6 ± 0.4 µg
Pseudomonas spp.	200 µl	9.0 ± 0.5 µg
Bacillus subtilis	1000 µl	5.7 ± 0.2 µg
Staphylococcus spp.	1000 µl	5.7 ± 0.2 µg

Bacterial DNA yield when purifying DNA from primary samples

When purifying bacterial DNA from primary sample material (e.g., nasopharyngeal secretions, tracheal secretions, tissue, blood, CSF, urine, and sputum samples), the yield of DNA depends on the sample type and the number of DNA-containing cells in the sample. Genomic DNA from contaminating host cells will co-purify with pathogen DNA.

Presence and yield of pathogens from primary samples are typically quantified by downstream analysis such as PCR or real-time PCR.

Protocol: Purification of DNA from Tissue

Select the appropriate EZ1 Card according to the EZ1 instrument you are using.

Table 4. Selection of EZ1 Card

Volume of sample	EZ1 Card required	Volume of eluted DNA
200 µl predigested sample	EZ1 Advanced XL DNA Tissue Card,* EZ1 Advanced DNA Tissue Card,† or EZ1 DNA Tissue 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&2 DNA Tissue Kit for the first time, read “Important Notes” (page 11).

- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In the case of liquid spillage, refer to “Safety Information” (page 6). Do not use damaged kit components, since their use may lead to poor kit performance.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 6 for safety information.
- In some steps of the procedure, one of two 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 (15–25°C).

Procedure

1. Transfer tissue into a 1.5 ml screw-capped tube (not supplied).

   For most tissue types, a sample size of 10 mg is recommended; however, for heart up to 20 mg and for muscle up to 40 mg may be used. See Table 3, page 21, for more information.
2. Add 190 µl Buffer G2.

   Ensure tissue pieces are fully submerged in Buffer G2.
3. Add 10 µl proteinase K solution and mix by tapping on the tube gently.
4. Incubate at 56°C until the tissue is completely lysed. Vortex 2–3 times per hour during incubation to disperse the sample, or place in an Eppendorf® Thermomixer, shaking water bath, or on a rocking platform.

   Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 3 h. Lysis overnight is possible and does not influence the preparation.
5. Homogenize the sample by pipetting up and down several times. Large pieces of insoluble material, which could clog pipette tips, should be removed by a quick centrifugation (300 x *g*, 1 min). Transfer the supernatant to a new 2 ml sample tube.
6. Insert ▲ the EZ1 Advanced DNA Tissue Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Tissue Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Tissue Card completely into the EZ1 Card slot of the BioRobot EZ1.
7. Switch on the EZ1 instrument.

8. 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.

9. ● Press **1** to start worktable setup for the Tissue Protocol.
10. Choose the elution volume: press **1** to elute in 50 µl, **2** to elute in 100 µl, or **3** to elute in 200 µl.
11. Proceed through the text shown on the display.

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

12. Open the instrument door.
13. 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.
14. Load the reagent cartridges into the cartridge rack.*†

    Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

15. Load opened elution tubes into the first row.†
16. Load tip holders containing filter-tips into the second row.†
17. Load opened sample tubes containing tissue samples into the fourth row.†
18. Close the instrument door.
19. Press **START** to start the purification procedure.

    The automated purification procedure takes approximately 20 min.

20. 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.

* See Figure 3B on page 16.
† See Figure 4 on page 17.

21. Open the instrument door.
22. Remove the elution tubes containing the purified DNA. Discard the sample preparation waste.*
23. ▲ **Optional**: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.
24. To run another protocol, press **ESC**, prepare samples as described in steps 1–5, and follow the procedure from step 9 onward. Otherwise, press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.
25. 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 page 6 for safety information

Protocol: Purification of DNA from Buccal Cells

Select the appropriate EZ1 Card according to the EZ1 instrument you are using.

Table 5. Selection of EZ1 Card

Volume of sample	EZ1 Card required	Volume of eluted DNA
200 µl proteinase K-digested sample	EZ1 Advanced XL DNA Buccal Swab Card,* EZ1 Advanced DNA Buccal Swab Card,† or EZ1 DNA Buccal Swab Card‡	100 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&2 DNA Tissue Kit for the first time, read “Important Notes” (page 11).

- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In the case of liquid spillage, refer to “Safety Information” (page 6). Do not use damaged kit components, since their use may lead to poor kit performance.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 6 for safety information.
- All steps of the DNA purification procedure should be performed at room temperature (15–25°C). During the setup procedure, work quickly.
- This protocol has been tested using the following swab types: plastic swabs with cotton or Dacron® tips. (Puritan® applicators with plastic shafts and cotton or Dacron tips are available from: Hardwood Products Company, **www.hwppuritan.com**, item nos. 25-806 1PC and 25-806 1PD; and from Daigger, **www.daigger.com**, cat. nos. EF22008D and EF22008DA). Nylon cytology brushes and other swab types may also be used.

- In some steps of the procedure, one of two 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

- Collect samples as described in "Buccal cells", page 11.
- The buffer in well 1 of the reagent cartridge (i.e., the well that is nearest to the front of the EZ1 instrument when the reagent cartridge is loaded) may form a precipitate upon storage. If necessary, redissolve by warming at 37°C and then place at room temperature (15–25°C).
- Before use, dilute Buffer G2 in distilled water using a ratio of 1:0.5 (i.e., one volume of Buffer G2 to 0.5 volumes of distilled water) for n+1 samples (where n is the number of samples to be digested). Buffer G2 may also be used undiluted although, due to the increased volume of Buffer G2 required for the buccal cells protocol, fewer isolations will be possible. Use of diluted Buffer G2 does not influence DNA yield or quality.

Procedure

1. Carefully cut or break off the end part of the swab or brush into a 2 ml sample tube (with screw cap), using an appropriate tool (e.g., scissors). Add 290 µl of diluted Buffer G2 to the sample.

   Note: Prepare diluted Buffer G2 as described above in "Things to do before starting".
2. Add 10 µl proteinase K, and mix thoroughly by vortexing for 10 s.

   If processing buccal cell brush samples, centrifuge the tube briefly (at 10,000 x *g* for 30 s) to force the brush to the bottom of the tube.
3. Incubate at 56°C for 15 min.

   Vortex the tube 1–2 times during the incubation, or place in a thermomixer.
4. Centrifuge the tube briefly to remove drops from inside the lid.

5. Remove the swab or brush from the tube.

   Using forceps, press the swab or brush against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200 µl.

6. Insert ▲ the EZ1 Advanced DNA Buccal Swab Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Buccal Swab Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Buccal Swab Card completely into the EZ1 Card slot of the BioRobot EZ1.

7. Switch on the EZ1 instrument.

8. 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.

9. ● Press **1** to start worktable setup.

10. Proceed through the text shown on the display.

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

11. Open the instrument door.

12. 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.

13. Load the reagent cartridges into the cartridge rack.*†

    Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

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

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

16. Load opened sample tubes containing buccal cell samples into the fourth row.†

17. Close the instrument door.

* See Figure 3B on page 16.
† See Figure 4 on page 17.

18. Press **START** to start the purification procedure.

    The automated purification procedure takes approximately 20 min.

19. 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.

20. Open the instrument door.

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

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

23. To run another protocol, press **ESC**, prepare samples, and follow the procedure from step 9 onward. Otherwise, press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

24. 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 page 6 for safety information.

Protocol: Purification of DNA from Cultured Cells

Select the appropriate EZ1 Card according to the EZ1 instrument you are using.

Table 6. Selection of EZ1 Card

Volume of sample	EZ1 Card required	Volume of eluted DNA
2 x 10^6 cells resuspended in 200 µl Buffer G2	EZ1 Advanced XL DNA Tissue Card,* EZ1 Advanced DNA Tissue Card,† or EZ1 DNA Tissue 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&2 DNA Tissue Kit for the first time, read "Important Notes" (page 11).

- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In the case of liquid spillage, refer to "Safety Information" (page 6). Do not use damaged kit components, since their use may lead to poor kit performance.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 6 for safety information.
- All steps of the DNA purification procedure should be performed at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of two choices can be made. Choose ▲ 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 (15–25°C).

Procedure

1. Centrifuge a maximum of 2 x 10^6 cells at 300 x *g* for 5 min in a 2 ml sample tube (with screw-cap) at room temperature (15–25°C).
2. Remove and discard the supernatant, taking care not to disturb the cell pellet.

   At this point, cells may be frozen (at –20°C or –70°C) for future purification or may be used immediately.
3. Add 200 µl Buffer G2 to the 2 ml sample tube containing approximately 2 x 10^6 cells. Resuspend the cells thoroughly by pipetting up and down.
4. Insert ▲ the EZ1 Advanced DNA Tissue Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Tissue Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Tissue 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. ● Press **1** to start worktable setup.
8. Choose the elution volume: press **1** to elute in 50 µl, **2** to elute in 100 µl, or **3** to elute in 200 µl.

9. Proceed through the text shown on the display.

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

10. Open the instrument door.
11. 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.
12. Load the reagent cartridges into the cartridge rack.* †

    Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

13. Load opened elution tubes into the first row.†
14. Load tip holders containing filter-tips into the second row.†
15. Load opened sample tubes containing cultured cell samples into the fourth row.†
16. Close the instrument door.
17. Press **START** to start the purification procedure.

    The automated purification procedure takes 15–20 min.

18. 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.

19. Open the instrument door.
20. Remove the elution tubes containing the purified DNA. Discard the sample preparation waste.‡
21. ▲ **Optional**: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.

* See Figure 3B on page 16.

† See Figure 4 on page 17.

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

22. 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.
23. Clean the EZ1 instrument.

    Follow the maintenance instructions in the user manual.

Protocol: Purification of DNA from Dried Blood

Select the appropriate EZ1 Card according to the EZ1 instrument you are using.

Table 7. Selection of EZ1 Card

Volume of sample	EZ1 Card required	Volume of eluted DNA
4 x 3 mm discs	EZ1 Advanced DNA Dried Blood Card,† or EZ1 DNA Dried Blood Card‡	50 µl, 100 µl, or 200 µl

† 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&2 DNA Tissue Kit for the first time, read "Important Notes" (page 11).

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

Procedure

1. Collect 70 µl of each blood sample onto a ring marked on filter paper. Allow the blood to air dry.

   Either untreated blood or blood containing anticoagulant (EDTA, ACD, or heparin) can be used.
2. For each dried blood sample, use the manual paper punch to cut out four 3 mm diameter discs.
3. Transfer each set of 4 discs to a 2 ml sample tube (provided in the kit).
4. Insert ▲ the EZ1 Advanced DNA Dried Blood Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or ● the EZ1 DNA Dried Blood 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. ● Press **1** to start worktable setup.
8. Choose the elution volume: press **1** to elute in 50 µl, **2** to elute in 100 µl, or **3** to elute in 200 µl.
9. Proceed through the text shown on the display.

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

10. Open the instrument door.
11. 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.
12. Load the reagent cartridges into the cartridge rack.* †

    Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.
13. Load opened elution tubes into the first row.†
14. Load tip holders containing filter-tips into the second row.†
15. Load opened sample tubes containing 4 discs of dried blood into the heating block.†

    Note: Be sure to load the samples into the heating block and NOT into the tip rack!
16. Close the instrument door.
17. Press **START** to start the purification procedure.

    The automated purification procedure takes approximately 40 min.
18. When the protocol ends, the display shows "Protocol finished". ▲ Press **ENT** to generate the report file.

    The EZ1 Advanced can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.
19. Open the instrument door.
20. Remove the elution tubes containing the purified DNA. Discard the sample preparation waste. ‡
21. ▲ **Optional**: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.

* See Figure 3B on page 16.

† See Figure 4 on page 17.

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

22. To run another protocol, press **ESC**, prepare samples as described in steps 1–3, 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.
23. Clean the EZ1 instrument.

    Follow the maintenance instructions in the user manual.

Protocol: Purification of DNA from Paraffin-Embedded Tissue

Select the appropriate EZ1 Card according to the EZ1 instrument you are using.

Table 8. Selection of EZ1 Card

Volume of sample	EZ1 Card required	Volume of eluted DNA
200 µl proteinase K-digested sample	EZ1 Advanced XL DNA Paraffin Section Card,* EZ1 Advanced DNA Paraffin Section Card,† or EZ1 DNA Paraffin Section 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&2 DNA Tissue Kit for the first time, read “Important Notes” (page 11).

- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In the case of liquid spillage, refer to “Safety Information” (page 6). Do not use damaged kit components, since their use may lead to poor kit performance.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 6 for safety information.
- All steps of the DNA purification procedure should be performed at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of two choices can be made. Choose ▲ 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 (15–25°C).
- Prepare a 75°C shaking water bath or thermomixer for use in step 3.

Procedure

1. Transfer sections of paraffin-embedded tissue into a 1.5 ml sample tube (not supplied). For most tissue types, a sample size of one to five 10 µm-thick sections is recommended; however, up to 10 very small sections such as needle biopsies may be used.
2. Add 190 µl Buffer G2.

   Ensure that the tissue sections are fully submerged in Buffer G2.
3. Incubate for 5 min at 75°C, with vigorous mixing (i.e., in a shaking water bath or thermomixer).
4. Lower the temperature to 56°C, and allow the sample to cool to 56°C.
5. Add 10 µl proteinase K solution, and mix by tapping the tube gently.
6. Incubate overnight at 56°C, with continuous vigorous mixing (i.e., in a shaking water bath or thermomixer).

   Note: To ensure complete digestion of the sample, overnight incubation is strongly recommended. Shorter incubation times may result in incomplete digestion of the sample, which may lead to clogging of tips during the automated DNA purification procedure.
7. Centrifuge the tube briefly to remove drops from inside the lid.
8. Homogenize the sample by pipetting up and down several times. Large pieces of insoluble material, which could clog pipette tips, should be removed by a quick centrifugation (300 x *g*, 1 min). Transfer the supernatant to a new 2 ml sample tube.

9. Insert ▲ the EZ1 Advanced DNA Paraffin Section Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Paraffin Section Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Paraffin Section Card completely into the EZ1 Card slot of the BioRobot EZ1.
10. Switch on the EZ1 instrument.
11. 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.
12. ● Press **1** to start worktable setup.
13. Choose the elution volume: press **1** to elute in 50 µl, **2** to elute in 100 µl, or **3** to elute in 200 µl.
14. Proceed through the text shown on the display.

    The text summarizes the following steps which describe the loading of the worktable.
15. Open the instrument door.
16. 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.
17. Load the reagent cartridges into the cartridge rack.* †

    Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.
18. Load opened elution tubes into the first row.†
19. Load tip holders containing filter-tips into the second row.†
20. Load opened sample tubes containing digested samples into the fourth row.†
21. Close the instrument door.

* See Figure 3B on page 16.
† See Figure 4 on page 17.

22. Press **START** to start the purification procedure.

    The automated purification procedure takes 15–20 min.

23. 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.

24. Open the instrument door.

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

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

27. To run another protocol, press **ESC**, prepare samples, and follow the procedure from step 12 onward. Otherwise, press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

28. 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 page 6 for safety information.

Protocol: Purification of Bacterial DNA from Primary Samples

Select the appropriate EZ1 Card according to the EZ1 instrument you are using.

Table 9. Selection of EZ1 Card

Volume of sample	EZ1 Card required	Volume of eluted DNA
200 µl	EZ1 Advanced XL DNA Bacteria Card,* EZ1 Advanced DNA Bacteria Card,† or EZ1 DNA Bacteria 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&2 DNA Tissue Kit for the first time, read "Important Notes" (page 11).

- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In the case of liquid spillage, refer to "Safety Information" (page 6). Do not use damaged kit components, since their use may lead to poor kit performance.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 6 for safety information.
- All steps of the protocol should be performed at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of two choices can be made. Choose ▲ 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 (15–25°C).
- If working with body fluid, secretion swabs, or viscous or mucous samples, preheat a thermomixer, heating block, or water bath to 56°C (body fluid or secretion swabs) or 37°C (viscous or mucous samples).
- If working with swabs, dilute Buffer G2 in distilled water at a ratio of 1:0.5 (i.e., one volume of Buffer G2 to 0.5 volumes of distilled water). The final volume should be sufficient for n+1 samples (where n is the number of samples to be digested). Buffer G2 may also be used undiluted. However, this would mean that fewer purifications could be performed because of the higher volume of Buffer G2 required for preparing swabs compared to other sample types. Use of diluted Buffer G2 does not influence DNA yield or quality.

Procedure

Preparation of sample material

Sample preparation requirements are highly dependent upon the type of starting material. Due to variations in consistency and viscosity, even similar sample types may require distinct handling. The steps below describe some recommendations for processing primary samples.

Body fluid and secretion swabs

1. Submerge the swab tip in 290 µl diluted Buffer G2 in a 1.5 ml or 2 ml tube. Add 10 µl proteinase K.
2. Incubate for 15 min at 56°C, with continuous mixing.

   If mixing is not possible, vortex before and after incubation.

3. Remove the swab and squeeze out all liquid by pressing the swab against the inside of the tube.
4. Transfer 200 µl of the sample to a 2 ml sample tube (supplied).

Viscous or mucous samples

1. If the sample is low viscosity, no preparation is needed. Transfer 200 µl sample into a 2 ml sample tube (supplied) and proceed to DNA purification.
2. If the sample is medium to high viscosity, dilute the sample 1:1 with 1x PBS in a 1.5 ml or 2 ml tube. Add freshly prepared DTT to a final concentration of 0.15% (w/v) (optional).
3. Incubate at 37°C until the sample can be pipetted.
4. Transfer 200 µl sample into a 2 ml sample tube (supplied).

Urine

5. Transfer urine sample into a 2 ml sample tube (supplied). 200 µl of urine can be used directly, without any preparation. Proceed to DNA purification.
6. If a more concentrated DNA sample is required, centrifuge 1 ml urine for 5 min at 3000 rpm.
7. Resuspend the cell pellet in 200 µl Buffer G2.

Stool

8. Add 500 µl 1x PBS to 50 mg of stool sample in a 1.5 ml or 2 ml tube (not supplied) and vortex for 1 min.
9. Incubate at room temperature (15–25°C) for 10 min.
10. Vortex the sample for 1 min.
11. Centrifuge for 30 s at 3000 rpm.

12. Transfer 200 µl of the liquid phase to a 2 ml sample tube (supplied).

Blood and blood culture

1. For Gram-negative bacteria, pretreatment of blood samples is not required. Transfer 200 µl blood or blood culture medium to a 2 ml sample tube (supplied).

   Proceed to DNA purification.
2. For Gram-positive bacteria, transfer 170 µl blood or blood culture medium to a 2 ml sample tube (supplied) and add 20 µl lysozyme (50 mg/ml) and/or 10 µl lysostaphin (5 mg/ml).
3. Incubate the sample at 37°C for 10 minutes.

DNA purification

1. Insert ▲ the EZ1 Advanced DNA Bacteria Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Bacteria Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Bacteria 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** to start worktable setup.
5. Choose the elution volume: press **1** to elute in 50 µl, **2** to elute in 100 µl, or **3** to elute in 200 µl.
6. Proceed through the text shown on the display.

   The text summarizes the following steps which describe the loading of the worktable.
7. Open the instrument door.

8. Invert reagent cartridges 4 times to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.
9. Load the reagent cartridges into the cartridge rack.*†

   Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.
10. Load opened elution tubes into the first row.†
11. Load tip holders containing filter-tips into the second row.†
12. Load opened sample tubes containing the samples into the fourth row.†
13. Close the instrument door.
14. Press **START** to start the purification procedure.

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

    The EZ1 Advanced and EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.
16. Open the instrument door.
17. Remove the elution tubes containing the purified DNA. Discard the sample preparation waste.‡
18. ▲ **Optional**: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.
19. To run another protocol, press **ESC**, prepare samples, and follow the procedure from step 4 onward. Otherwise, press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.

* See Figure 3B on page 16.

† See Figure 4 on page 17.

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

20. Clean the EZ1 instrument.

    Follow the maintenance instructions in the user manual.

Protocol: Purification of DNA from Bacterial Culture Samples

Select the appropriate EZ1 Card according to the EZ1 instrument you are using.

Table 10. Selection of EZ1 Card

Volume of sample	EZ1 Card required	Volume of eluted DNA
200 µl	EZ1 Advanced XL DNA Bacteria Card,* EZ1 Advanced DNA Bacteria Card,† or EZ1 DNA Bacteria 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&2 DNA Tissue Kit for the first time, read "Important Notes" (page 11).

- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In the case of liquid spillage, refer to "Safety Information" (page 6). Do not use damaged kit components, since their use may lead to poor kit performance.
- Proteinase K is not required for this protocol.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 6 for safety information.
- All steps of the DNA purification procedure should be performed at room temperature (15–25°C). During the setup procedure, work quickly.
- In some steps of the procedure, one of two choices can be made. Choose ▲ 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 (15–25°C).

Procedure

Pretreatment of bacterial cultures

Gram-negative bacteria

1. Centrifuge 200 µl of bacterial day-culture or overnight-culture for 5 min at 5000 x g and remove the supernatant. Alternatively, use several bacterial colonies. Resuspend the bacterial pellet or colonies in 200 µl Buffer G2 in a 2 ml sample tube (supplied).
2. For some bacterial species, up to 1 ml culture (resuspended in 200 µl) can be used.

   Note: It is often possible to purify DNA directly from 200 µl of bacterial culture without centrifugation.
3. To purify bacterial DNA, proceed to the DNA purification procedure.

Gram-positive bacteria

1. Centrifuge 200 µl of bacterial day-culture or overnight-culture for 5 min at 5000 x g and remove the supernatant. Alternatively, pick several bacterial colonies.

   For some bacterial species, up to 1 ml culture can be used.
2. Resuspend the bacterial pellet or colony in 180 µl Buffer G2 and transfer to a 2 ml sample tube (supplied).

3. **Optional**: Add 0.04 g of glass beads (≤106 µm) and vortex for 1–3 min. Do not remove the glass beads before DNA purification.

   Note: This step may provide superior DNA yield for bacterial strains with rigid cell walls.

4. Add 20 µl lysozyme (50 mg/ml) and/or 10 µl lysostaphin (5 mg/ml), and incubate the sample at 37°C for 30 min.

   Note: The duration of digestion can be extended to 2 hours for complete cell lysis.

5. Proceed to the DNA purification procedure.

DNA purification

1. Insert ▲ the EZ1 Advanced DNA Bacteria Card completely into the EZ1 Advanced Card slot of the EZ1 Advanced or the EZ1 Advanced XL DNA Bacteria Card completely into the EZ1 Advanced XL Card slot of the EZ1 Advanced XL or ● the EZ1 DNA Bacteria 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** to start worktable setup.
5. Choose the elution volume: press **1** to elute in 50 µl, **2** to elute in 100 µl, or **3** to elute in 200 µl.
6. Proceed through the text shown on the display.

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

7. Open the instrument door.
8. Invert reagent cartridges 4 times to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.

9. Load the reagent cartridges into the cartridge rack.*†

   Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

10. Load opened elution tubes into the first row.†
11. Load tip holders containing filter-tips into the second row.†
12. Load opened sample tubes containing the samples into the fourth row.†
13. Close the instrument door.
14. Press **START** to start the purification procedure.

    The automated purification procedure takes 15–20 min.

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

    The EZ1 Advanced and EZ1 Advanced XL can store up to 10 report files. Report files can be printed directly on a connected printer or transferred to a computer.

16. Open the instrument door.
17. Remove the elution tubes containing the purified DNA. Discard the sample preparation waste.‡
18. ▲ **Optional**: Follow the onscreen instructions to perform UV decontamination of the worktable surfaces.
19. To run another protocol, press **ESC**, prepare samples, and follow the procedure from step 4 onward. Otherwise, press **STOP** twice to return to the first screen of the display, close the instrument door, and switch off the EZ1 instrument.
20. Clean the EZ1 instrument.

    Follow the maintenance instructions in the user manual.

* See Figure 3B on page 16.

† See Figure 4 on page 17.

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

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page 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 whether the printer is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port. Check whether the serial port is set for use with a printer.
c) Report file not sent to the PC	Check whether the PC is connected to the EZ1 Advanced or EZ1 Advanced XL via the "PC/Printer" serial port. Check whether the serial port is set for use with a PC.
d) Wrong Q-Card ID entered	If the wrong ID was entered instead of the Q-Card ID, the EZ1 Advanced or EZ1 Advanced XL will not accept the ID and will prompt for the Q-Card ID until the correct ID is entered. Press **STOP** twice to go to the main menu.
Low DNA yield	
a) Magnetic particles not completely resuspended	Ensure that you invert the reagent cartridges several times to resuspend the magnetic particles.
b) Insufficient reagent aspirated	After inverting the reagent cartridges to resuspend the magnetic particles, ensure that you tap the cartridges to deposit the reagents at the bottom of the wells.

	Comments and suggestions
c) Varying pipetting volumes	To ensure pipetting accuracy, it is important that buffer volumes in the reagent cartridges are correct and that the filter tips fit optimally to the tip adapter. Ensure that samples are thoroughly mixed and that reagent cartridges have not passed their expiry date. Perform regular maintenance as described in the instrument user manual. Check the fit of the filter tips regularly as described in the user manual.
d) Precipitates in cartridges and buffers	Make sure the extraction room temperature is not too cold; temperatures at ~23/24°C may help to prevent precipitation. Do a careful visual inspection of the cartridges. Warm EZ1 cartridges. Use the sample lysate when it is still hot from the incubator.

High A_{230} value

Spectrophotometer calibrated with water	To obtain accurate quantification of purified DNA using a spectrophotometer, the instrument must be calibrated with a suitable dilution buffer.

DNA does not perform well in downstream applications

a) Insufficient DNA used in downstream application	Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see "Quantification of DNA", page 55).
b) Excess DNA used in downstream application	Excess DNA can inhibit some enzymatic reactions. Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see "Quantification of DNA", page 55).

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

Absorbance reading at 320 nm not subtracted from the absorbance readings obtained at 260 nm and 280 nm	To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see "Quantification of DNA", page 55).

Low DNA yield from buccal cells

Insufficient number of cells in sample	When collecting buccal cell samples, be sure to scrape the swab or brush firmly against the inside of each cheek 6 times.

Low DNA yield from cultured cells

Clogging due to sample overload	Too many cells may have been used. Ensure that no more than 2 x 10^6 cells are used per purification.

Comments and suggestions	
Low bacterial DNA purity from primary samples	
Sample overload	Follow the recommendations in "Preparation of sample material" page 43.
Clumping of beads when working with primary samples	
High sample viscosity or sample overload	Follow the recommendations in "Preparation of sample material" page 43.
Poor amplification sensitivity after bacterial DNA purification from primary samples	
a) Inhibition of amplification	Add an internal control to the sample. Reduce the amount of sample material. Increase the elution volume.
b) Inefficient lysis	For Gram-positive bacteria, pretreat the sample with lysozyme and/or lysostaphin as described in "Blood and blood culture", page 45. If necessary, extend the incubation time up to 2 h.
Low DNA purity from bacterial culture	
Sample overload	Reduce the amount of sample material.
Clumping of beads when working with bacterial culture	
Sample overload	Reduce the amount of sample material.
Poor amplification sensitivity after DNA purification from bacterial culture	
a) Inhibition of amplification	Reduce the amount of sample material. Increase the elution volume.
b) Inefficient lysis	Follow the "Pretreatment of bacterial cultures" procedure, page 49. Increase the amount of lysozyme and the duration of digestion.

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

Storage of DNA

Purified DNA may be stored at 2–8°C for 24 hours or at –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). Carryover of magnetic particles in the eluate may affect the A_{260} reading, but should not affect the performance of the DNA in downstream applications. If the purified DNA is to be analyzed by fluorescent capillary sequencing, the tube containing the eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see below).

To quantify DNA isolated using the EZ1 system:

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

  Note: The elution buffer in the Reagent Cartridge, Tissue contains UV-active sodium azide. This must be taken into account when calibrating the spectrophotometer:

- For undiluted samples, use the supplied Buffer AVE to calibrate the spectrophotometer (pure).
- If the samples need to be diluted, use buffer of slightly alkaline pH (e.g., 10 mM Tris·Cl, pH 7.5) to dilute the samples. In this case, use the same dilution of the supplied Buffer AVE in the chosen dilution buffer for calibration of 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_{260} – A_{320}). Pure DNA has an A_{260}/A_{280}) ratio of 1.7–1.9.

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 Tissue

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: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801201
Reagent Lot number: _ _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ Dec 14, 2008
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 848373922
Note: _ 2000

Channel B:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 234567890
Reagent Kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801201
Reagent Lot number: _ _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ Dec 14, 2008
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 836266738
Note: _

Channel C:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 345678901
Reagent Kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801201
Reagent Lot number: _ _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ Dec 14, 2008
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 883727832
Notes: _ 1000

Channel D:
Sample ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 456789012
Reagent Kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ 9801201
Reagent Lot number: _ _ _ _ _ _ _ _ _ _ _ _ _ 23456789
Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ Dec 14, 2008
Assay kit ID: _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ 763684837
Note: _

Channel E:

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

Reagent Kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 9801201

Reagent Lot number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 23456789

Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ Dec 14, 2008

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

Note: _

Channel F:

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

Reagent Kit number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 9801201

Reagent Lot number: _ _ _ _ _ _ _ _ _ _ _ _ _ _ 23456789

Reagent Expiry date: _ _ _ _ _ _ _ _ _ _ Dec 14, 2008

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

Note: _ 50

Ordering Information

Product	Contents	Cat. no.
EZ1&2 DNA Tissue Kit (48)	48 Reagent Cartridges (Tissue), 50 Disposable Tip Holders, 50 Disposable Filter-Tips, 50 Sample Tubes (2 ml), 50 Elution Tubes (1.5 ml), Buffer G2, Proteinase K, Buffer AVE	953034
EZ2 Connect	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
Accessories		
EZ1 Advanced DNA Tissue Card	Preprogrammed card for EZ1 Advanced DNA tissue protocols	9018295
EZ1 Advanced XL DNA Tissue Card	Preprogrammed card for EZ1 Advanced XL DNA tissue protocols	9018701
EZ1 Advanced DNA Buccal Swab Card	Preprogrammed card for EZ1 Advanced DNA buccal swab protocols	9018296
EZ1 Advanced XL DNA Buccal Swab Card	Preprogrammed card for EZ1 Advanced XL DNA buccal swab protocols	9018696
EZ1 Advanced DNA Dried Blood Card	Preprogrammed card for EZ1 Advanced DNA dried blood protocols	9018299
EZ1 Advanced DNA Paraffin Section Card	Preprogrammed card for EZ1 Advanced DNA paraffin section protocols	9018298

Product	Contents	Cat. no.
EZ1 Advanced XL DNA Paraffin Section Card	Preprogrammed card for EZ1 Advanced XL DNA paraffin section protocols	9018700
EZ1 Advanced DNA Bacteria Card	Preprogrammed card for EZ1 Advanced DNA bacteria protocols	9018301
EZ1 Advanced XL DNA Bacteria Card	Preprogrammed card for EZ1 Advanced XL DNA bacteria protocols	9018694
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

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Document Revision History

Date	Changes
11/2021	Rebranded the name of the kit from “EZ1” to “EZ1&2”. Added the EZ2 Connect Instrument to the “Ordering Information” section. Updated the cleaning procedure. Editorial and layout changes.
08/2022	Increased volumes for Buffer G2 (page 4), adjusted Troubleshooting Guide for “Precipitates in cartridges and buffers”.

Notes

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