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EZ1&2™ DNA Tissue Handbook

EZ1&2 DNA Tissue Kit
For automated purification of DNA from tissue and other samples using EZ2® Connect instruments

Sample to Insight

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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 11 mL
Proteinase K	2 x 250 µL
Buffer AVE	2 mL
Q-Card*	1
Handbook	1

* The information encoded in the bar code on the Q-Card is needed for reagent data tracking using the EZ2 Connect 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 	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 EZ2 Connect instrument, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite, followed by water.

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. Magnetic-particle technology provides high-quality DNA that is suitable for direct use in downstream applications such as amplification or other enzymatic reactions. The EZ2 Connect 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 24 samples are processed in a single run.

This handbook refers to processing of EZ1&2 DNA Tissue Kit with EZ2 Connect instruments. For usage of the EZ1&2 DNA Tissue Kit with EZ1 instruments, please refer to the respective handbook (**www.qiagen.com/HB-0196**) and quick-start protocol (**www.qiagen.com/HB-0784**).

Principle and procedure

Magnetic-particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (see the workflow below). 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.

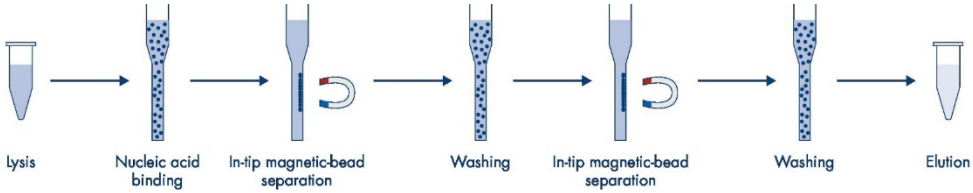

Figure 1. EZ1&2 DNA Tissue Kit workflow.

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 Tissue Protocol

- 1.5 mL screw-capped tube

For Buccal Cells Protocol

- Swabs (for swab types tested with the protocol, see “Things to do before starting”, page 20).

For Dried Blood Protocol

- Filter paper (e.g., QIAcard® FTA® Spots, see “Ordering Information”, page 39)
- 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 28
- 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

- 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.
- The buffer in well 1 of the reagent cartridge (i.e., the well that is nearest to the front of the EZ2 Connect 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).
- All steps of the DNA purification procedure should be performed at room temperature (15–25°C). During the setup procedure, work quickly.

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 2, page 17)	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 10^6 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
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 hours after collection. Ensure that the person providing the sample has not consumed any food or drink for 30 minutes prior to sample collection.

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 EZ2 Connect 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 (A_{260}) of the purified DNA but should not affect downstream applications. The measured absorbance at 320 nm (A_{320}) should be subtracted from all absorbance readings. See "Quantification of DNA", page 37, for more information.

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

Working with EZ2 Connect instruments

The main features of EZ2 Connect instruments include:

- Purification of high-quality nucleic acids from up to 24 samples per run
- Small footprint to save laboratory space
- Preprogrammed protocols for nucleic acid purification
- Prefilled, sealed reagent cartridges for easy, safe, and fast run setup
- Complete automation of nucleic acid purification, from opening of reagent cartridges to elution of nucleic acids, with no manual centrifugation steps
- Optional bar code reading and sample tracking
- Kit data tracking with the Q-Card provided in the kit
- UV LED 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 EZ2 Connect. 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.

EZ2 Connect reagent cartridges

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (Figure 2). Each well of the cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Positions 11 and 12 can be equipped individually. Details on preparation of these positions are displayed during the run setup on the LED display of the EZ2 Connect.

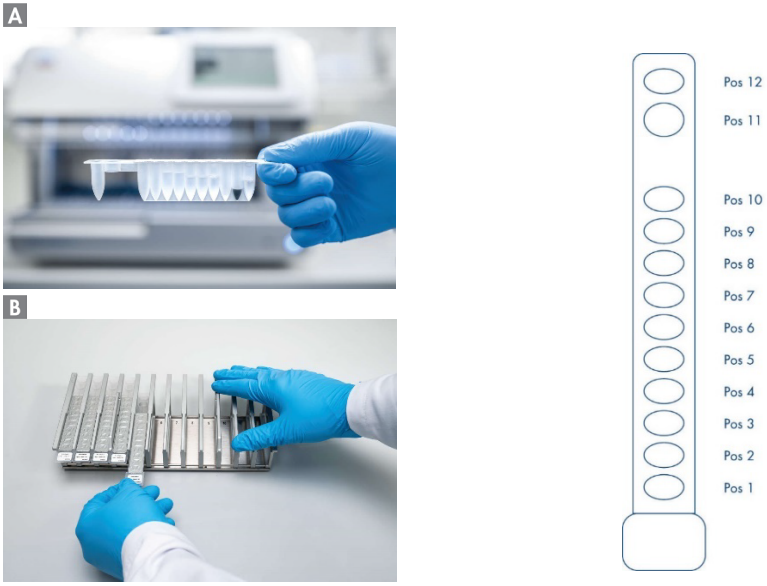

Figure 2. Ease of worktable setup using reagent cartridges. (A) A sealed, prefilled reagent cartridge. Fill levels vary, depending on the type of reagent cartridge. (B) Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded. Each reagent cartridge contains 12 individual positions.

EZ2 Connect tip racks

The EZ2 Connect tip racks holds tips inserted into tip holders and tubes for samples or elution. Details on how to equip the tip racks are displayed during the run setup on the LED display of the EZ2 Connect.

Figure 3. The EZ2 Connect Tip Rack (A) has 4 positions label A–D by engravings. It is designed to hold sample and elution tubes as well as tips in their respective tip holders (B).

Worktable

The worktable of EZ2 Connect instruments is where the user equips cartridge and tip racks (Figure 4). The display also shows protocol status during the automated purification procedure.

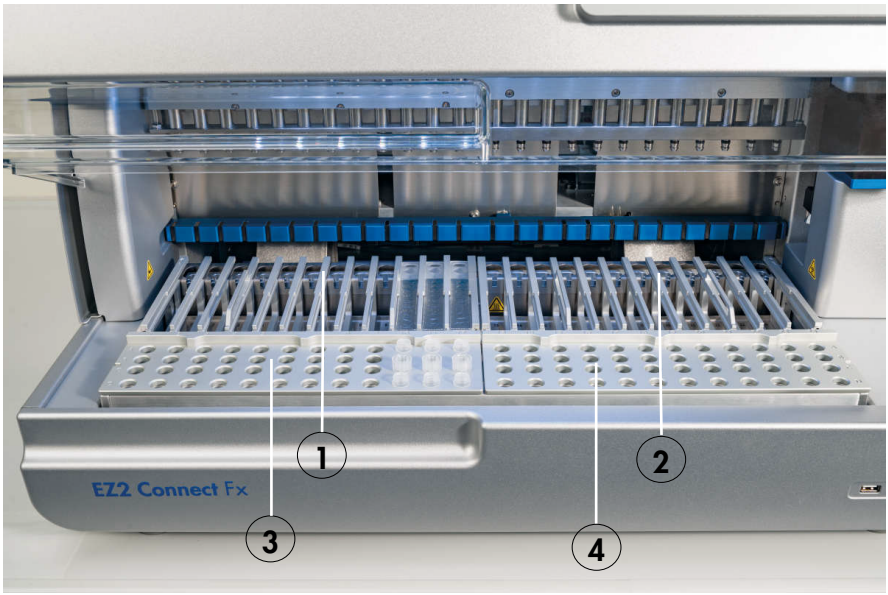

Figure 4. EZ2 Connect Worktable.
1. EZ2 Connect Cartridge Rack – left
2. EZ2 Connect Cartridge Rack – right
3. EZ2 Connect Tip Rack – left
4. EZ2 Connect Tip Rack – right

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*.

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 2 shows typical yields obtained from different sample volumes and sample types.

Table 2. 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

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 2, page 17 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 a 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 impair the preparation.

5. Homogenize the sample by pipetting up and down several times. Large pieces of insoluble material should be removed by a quick centrifugation (300 x *g*, 1 min). Transfer the supernatant to a new 2 mL sample tube.

6. Turn on the EZ2 Connect instrument.

7. Tap **DNA** on the Applications panel and then select **DNA Tissue** and press **Next**.

8. Choose the elution volume and press **Next**.

9. Select positions on the work deck according to the number of samples to be processed and press **Next**.

10. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.

11. Load the EZ1&2 Tissue Mini reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 9. Press **Next**.

12. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.
13. Prepare the EZ2 Connect Tip Rack as follows (Figure 3):
    - Position A: Opened 2.0 mL tube with sample
    - Position B: Empty
    - Position C: Tip holder with inserted tip
    - Position D: Opened 1.5 mL empty elution tube

    Press **Next**.
14. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.
15. The display will show "Protocol finished" when the run is completed. Select **Finish**.
16. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position 1 of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste.

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

Protocol: Purification of DNA from Buccal Cells

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.

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 EZ2 Connect 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. Turn on the EZ2 Connect instrument.
7. Tap **DNA** on the Applications panel and then select **DNA Buccal Swab** and press **Next**.
8. Choose the elution volume and press **Next**.
9. Select positions on the work deck according to the number of samples to be processed and press **Next**.
10. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
11. Load the EZ1&2 Tissue Mini reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 9. Press **Next**.
12. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.
13. Prepare the EZ2 Connect Tip Rack as follows (Figure 3):
    - Position A: Opened 2.0 mL tube with sample
    - Position B: Empty
    - Position C: Tip holder with inserted tip
    - Position D: Opened 1.5 mL empty elution tube

    Press **Next**.

14. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.
15. The display will show “Protocol finished” when the run is completed. Select **Finish**.

16. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position 1 of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste.

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

17. Perform regular maintenance after each run. Press **Finish** to return to the home screen.

Protocol: Purification of DNA from Cultured Cells

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. Turn on the EZ2 Connect instrument.
5. Tap **DNA** on the Applications panel and then select **DNA Tissue** and press **Next**.
6. Choose the elution volume and press **Next**.
7. Select positions on the work deck according to the number of samples to be processed and press **Next**.
8. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
9. Load the EZ1&2 Tissue Mini reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 7. Press **Next**.
10. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.
11. Prepare the EZ2 Connect Tip Rack as follows (Figure 3):
    - Position A: Opened 2.0 mL tube with sample
    - Position B: Empty
    - Position C: Tip holder with inserted tip
    - Position D: Opened 1.5 mL empty elution tube

    Press **Next**.

12. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.
13. The display will show “Protocol finished” when the run is completed. Select **Finish**.
14. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position 1 of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste.

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

Protocol: Purification of DNA from Dried Blood

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. Turn on the EZ2 Connect instrument.
5. Tap **DNA** on the Applications panel and then select **DNA Dried Blood** and press **Next**.
6. Choose the elution volume and press **Next**.
7. Select positions on the work deck according to the number of samples to be processed and press **Next**.
8. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
9. Load the EZ1&2 Tissue Mini reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 7. Load the 2 mL sample tube from step 3 into position 11 of reagent cartridge

   Press **Next**.
10. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.

11. Prepare the EZ2 Connect Tip Rack as follows (Figure 3):
    - Position A: Empty
    - Position B: Empty
    - Position C: Tip holder with inserted tip
    - Position D: Opened 1.5 mL empty elution tube

    Press **Next**.
12. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.
13. The display will show "Protocol finished" when the run is completed. Select **Finish**.
14. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position 1 of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste.

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

Protocol: Purification of Bacterial DNA from Primary Samples

If working with swabs, dilute Buffer G2 in distilled water at a ratio of 1:0.5 (i.e., 1 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.

   Optional: Add freshly prepared DTT to a final concentration of 0.15% (w/v).
3. Incubate at 37°C until the sample can be pipetted.
4. Transfer 200 µL sample into a 2 mL sample tube (supplied). Proceed to DNA purification.

Urine

1. 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.
2. If a more concentrated DNA sample is required, centrifuge 1 mL urine for 5 min at 3000 rpm.
3. Resuspend the cell pellet in 200 µL Buffer G2. Proceed to DNA purification.

Stool

1. 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.
2. Incubate at room temperature (15–25°C) for 10 min.
3. Vortex the sample for 1 min.
4. Centrifuge for 30 s at 3000 rpm.
5. Transfer 200 µL of the liquid phase to a 2 mL sample tube (supplied). Proceed to DNA purification.

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 mins. Proceed to DNA purification.

DNA purification

1. Turn on the EZ2 Connect instrument.
2. Tap **DNA** on the Applications panel and then select **DNA Bacteria** and press **Next**.
3. Choose the elution volume and press **Next**.
4. Select positions on the work deck according to the number of samples to be processed and press **Next**.
5. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
6. Load the EZ1&2 Tissue Mini reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 4. Press **Next**.
7. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.
8. Prepare the EZ2 Connect Tip Rack as follows (Figure 3):
   - Position A: Opened 2.0 mL tube with sample
   - Position B: Empty
   - Position C: Tip holder with inserted tip
   - Position D: Opened 1.5 mL empty elution tube

   Press **Next**.

9. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.
10. The display will show “Protocol finished” when the run is completed. Select **Finish**.
11. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position 1 of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste.

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

Protocol: Purification of DNA from Bacterial Culture Samples

Proteinase K is not required for this protocol.

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).

   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.

3. 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 h for complete cell lysis.

4. Proceed to the DNA purification procedure.

DNA purification

1. Turn on the EZ2 Connect instrument.
2. Tap **DNA** on the Applications panel and then select **DNA Bacteria** and press **Next**.
3. Choose the elution volume and press **Next**.
4. Select positions on the work deck according to the number of samples to be processed and press **Next**.
5. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
6. Load the EZ1&2 Tissue Mini reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 4. Press **Next**.
7. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.
8. Prepare the EZ2 Connect Tip Rack as follows (Figure 3):
   - Position A: opened 2.0 mL tube with sample
   - Position B: empty
   - Position C: tip holder with inserted tip
   - Position D: opened 1.5 mL empty elution tube

   Press **Next**.
9. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.
10. The display will show “Protocol finished” when the run is completed. Select **Finish**.

11. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position 1 of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste.

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

12. Perform regular maintenance after each run. Press **Finish** to return to the home screen.

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 EZ2 Connect instrument.
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.
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.
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 37).

	Comments and suggestions
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 37).

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 37).

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.

Low bacterial DNA purity from primary samples

Sample overload	Follow the recommendations in "Preparation of sample material" page 27.

Clumping of beads when working with primary samples

High sample viscosity or sample overload	Follow the recommendations in "Preparation of sample material" page 27.

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 29. If necessary, extend the incubation time up to 2 h.

	Comments and suggestions
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 31. 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 milliliter (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 EZ2 Connect instrument:

- 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.

To calibrate 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.

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
Related products		
Filter-Tips and Holders, EZ1 (50)	50 Disposable Filter-Tips, 50 Disposable Tip Holders; additional tips and holders for use with EZ1, EZ1&2 and EZ2 Kits	994900
QIAcard FTA One Spot (100)	For collection and storage of 100 samples: 100 QIAcard FTA One Spots	159201
QIAcard FTA Two Spots (100)	For collection and storage of 100 x 2 samples: 100 QIAcard FTA Two Spots	159203
QIAcard FTA Four Spots (100)	For collection and storage of 100 x 4 samples: 100 QIAcard FTA Four Spots	159205
QIAcard FTA Indicator Four Spots (25)	For collection and storage of 25 x 4 samples: 25 QIAcard FTA Indicator Four Spots	159214

QIAcard FTA Purification Reagent (500 mL)	For use with QIAcard FTA Spots: 500 mL QIAcard FTA Purification Reagent	159300

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

Document Revision History

Revision	Description
November 2022	Initial revision

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