April 2022

EZ1&2™ DNA FFPE Handbook

EZ1&2 DNA FFPE and EZ1&2 DNA FFPE UNG Kits

For automated purification of genomic DNA from formalin-fixed, paraffin-embedded (FFPE) tissues using EZ2® Connect instruments

Sample to Insight

QIAGEN

Contents

Kit Contents

Kit Name **Catalog no.** **No. of reactions**	**EZ1&2 DNA FFPE Kit (48)** **954404** **48**	**EZ1&2 DNA FFPE UNG Kit (48)** **954414*** **48**
Uracil-N-Glycosylase		2 x 1 ml
Paraffin Removal Solution	20 ml	20 ml
Buffer FTB	2 x 0.8 ml	2 x 0.8 ml
Proteinase K	2 x 1.25 ml	2 x 1.25 ml
RNase-Free Water	2 x 7 ml	2 x 7 ml
RNase A (100 mg/ml)	2 x 15 mg	2 x 15 mg
EZ1&2 DNA FFPE Cartridges†‡	48	48
Disposable Tip Holders	50	50
Disposable Filter-Tips	50	50
Tubes 1.5 ml	3 x 50	3 x 50
Tubes 2 ml	50	50
Buffer AVE‡	1.9 ml	1.9 ml
Q-Card§	1	1
Quick-Start Protocol	1	1

***** The EZ1&2 DNA FFPE UNG Kit (cat. no. 954414) consists of 2 items: the EZ1&2 DNA FFPE Kit (cat. no. 954404) and the Uracil-N-Glycosylase (UNG, cat. no. 19160).

† Contains chaotropic salt. Not compatible with disinfecting agents containing bleach; see page 5 for Safety Information.

‡ Contains sodium azide as a preservative.

§ The information encoded in the bar code on the Q-Card is needed for reagent data tracking using the EZ2 Connect instruments.

Shipping and Storage

The EZ1&2 DNA FFPE UNG Kit (cat. no. 954414) consists of the EZ1&2 DNA FFPE Kit (cat. no. 954404) and the Uracil-N-Glycosylase (UNG, cat. no. 19160).

Uracil-N-Glycosylase (UNG) is shipped on dry ice. It should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. Under these conditions, UNG is stable until the expiration date printed on the UNG tube label.

The EZ1&2 DNA FFPE Kit is shipped at ambient temperature. Buffers and reagents can be stored at room temperature (15–25°C). Do not freeze the reagent cartridges.

When stored properly, (buffers and) reagent cartridges are stable until the expiration date on the Q-Card and the kit label.

The EZ1&2 DNA FFPE Kit and the EZ1&2 DNA FFPE UNG Kit contain a ready-to-use Proteinase K solution, which is supplied in a specially formulated storage buffer. Proteinase K is stable for at least 1 year after delivery when stored at room temperature or if ambient temperatures often exceed 25°C, we suggest storing Proteinase K at 2–8°C.

Intended Use

The EZ1&2 DNA FFPE and the EZ1&2 DNA FFPE UNG kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of 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.

The EZ1&2 DNA FFPE and the EZ1&2 DNA FFPE UNG kits are intended to be used on EZ1 Advanced XL or EZ2 Connect instruments, including EZ2 Connect, EZ2 Connect Fx, and EZ2 Connect MDx.

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 EZ1&2 DNA FFPE cartridge contain chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a 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, please refer to the instrument user manual for decontamination instructions.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, the components of the EZ1&2 DNA FFPE UNG and EZ1&2 DNA FFPE kits are tested against predetermined specifications to ensure consistent product quality.

Introduction

Exposure to formalin results in cross-linkage and thereby stabilization of proteins and DNA. Formalin fixation followed by paraffin embedding of tissue specimens is a standard method for preserving histological structures within tissues. In addition, the resulting formalin-fixed, paraffin-embedded (FFPE) tissue samples are valuable for molecular analyses. However, DNA preparation from FFPE tissue is associated with several challenges. Yields are often low due to the limited availability of input material and the compromised quality of DNA due to fixation and long-term storage of samples. Recovery of amplifiable DNA strongly depends on removal of formalin-induced cross-links. The EZ1&2 DNA FFPE Kits include multiple steps to lyse fixed tissue and remove DNA cross-links.

Additionally, DNA sequence artifacts may be introduced by fixation, embedding, and long-term storage. The most common artifact in FFPE tissues is the deamination of cytosine bases to uracil. This leads to a C-T conversion during amplification, and a false result in downstream analysis. These artifacts can lead to false results when using sensitive methods for mutation analyses such as Next-Generation Sequencing (NGS) or digital PCR (dPCR) with limited starting material. The EZ1&2 DNA FFPE UNG procedure includes steps to remove deaminated cytosine bases to prevent these false results in DNA sequencing analyses.

The EZ1&2 DNA FFPE Kit and the EZ1&2 DNA FFPE UNG Kit provide convenient, streamlined procedures for efficient purification of high amounts of amplifiable DNA from difficult-to-lyse FFPE tissue sections.

Principle and procedure

This protocol version describes usage of the EZ1&2 DNA FFPE Kit on the EZ2 Connect instrument. For more information about use with the EZ1 Advanced XL, please refer to the EZ1&2 DNA FFPE Quick-Start Protocol (**www.qiagen.com/HB-2852**) and handbook (**www.qiagen.com/HB-2867**) for usage with EZ1 instruments.

The EZ1&2 DNA FFPE and EZ1&2 DNA FFPE UNG procedures remove paraffin without use of hazardous solvents and without the need to trim off excess paraffin from the FFPE block in advance. Formalin-induced cross-links are efficiently removed from the DNA. Two Proteinase K digestion steps, one before and one after DNA de-crosslinking, ensure complete lysis of even difficult-to-lyse tissue and facilitate the recovery of high amounts of amplifiable DNA.

After the initial Proteinase K digestion and incubation at elevated temperature to remove cross-links, dilution of the reaction mixture provides conditions that allow the optional removal of deaminated cytosine residues by the enzyme Uracil-N-Glycosylase (UNG). Prior to DNA binding, a second Proteinase K digestion step improves lysis efficiency and increases yields, particularly for difficult-to-lyse samples. DNA is then bound to magnetic particles. Contaminants that may interfere with subsequent enzymatic reactions are removed in different washing steps.

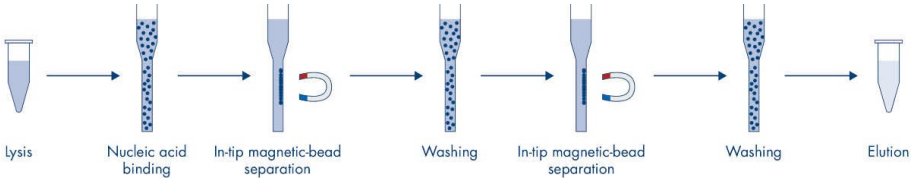
DNA is eluted in Buffer AVE. A spare tube of Buffer AVE is included in the kit to be used as control in downstream applications. Isolated DNA is compatible with PCR, digital PCR, and NGS workflows. If necessary, DNA can be stored long term at –30 to –15°C.

Automation

This protocol describes the workflow when using the EZ2 Connect instruments. For use with the EZ2 Connect instruments, two workflow options are available (see Figure 1). The EZ2 Connect instruments can perform all steps following deparaffinization of the sample in an automated procedure. Alternatively, steps prior to DNA binding such as lysis with Proteinase K, cross-link removal, RNase A digestion, and optional UNG treatment can be carried out manually. DNA binding, washing, and elution steps are then conducted on the EZ2 Connect instrument. Up to 24 samples may be processed in a single run.

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



Starting material

Typical formalin-fixation and paraffin-embedding procedures result in significant fragmentation of nucleic acids. To limit the extent of nucleic acid fragmentation, please use following guidelines:

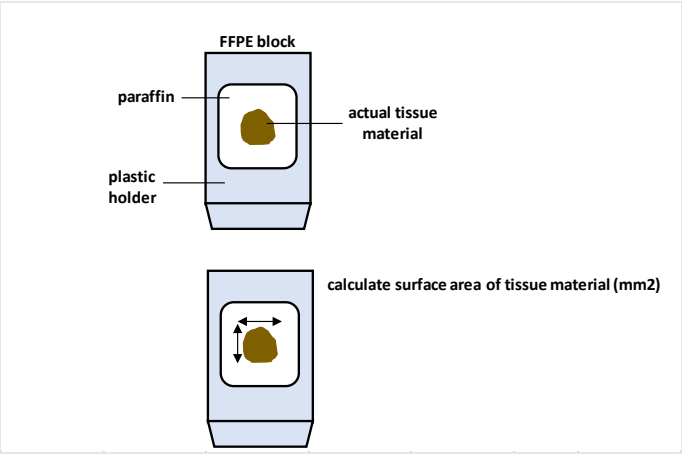
- Fix tissue samples in 4%–10% formalin as quickly as possible after specimen collection .
- Keep formalin fixation time to minimum (longer fixation times lead to more severe DNA fragmentation, resulting in poor performance in downstream assays).
- Thoroughly dehydrate samples after fixation. This will also remove residual formalin that can inhibit Proteinase K digestion.

Sample material for DNA extraction from FFPE tissue is prepared as 5 to 10 µm sections cut from a FFPE block using a microtome.

The amount of starting material specified for use with the EZ1&2 DNA FFPE Kit and EZ1&2 DNA FFPE UNG Kit refers to the actual tissue material of the FFPE sample, excluding the area of paraffin. The starting material is calculated from the surface area of the tissue, the number of sections, and the thickness of sections. With the EZ1&2 DNA FFPE Kit and EZ1&2 DNA FFPE UNG Kit, FFPE tissue sections of 5–10 µm thickness can be processed, totaling up to 4

mm^3 of tissue. In cases where calculating the exact amount is impossible, use no more than 2 sections of 5–10 µm thickness.

Sample volume and calculation



Surface area	No. of sections	Total volume
50 mm^2	1 section of 10 µm thickness	0.5 mm^3
	2 sections of 10 µm thickness	1 mm^3
	4 sections of 10 µm thickness	2 mm^3
	8 sections of 10 µm thickness	4 mm^3
100 mm^2	1 section of 10 µm thickness	1 mm^3
	2 sections of 10 µm thickness	2 mm^3
	4 sections of 10 µm thickness	4 mm^3
200 mm^2	1 section of 10 µm thickness	2 mm^3
	2 sections of 10 µm thickness	4 mm^3
400 mm^2	1 section of 10 µm thickness	4 mm^3

DNA quality and yield

FFPE tissue material presents challenges not only for the DNA extraction method itself but also for the determination of DNA quality and quantity. Generally, DNA yield from FFPE samples varies greatly, depending on the tissue type, as well as fixation and embedding conditions.

Furthermore, due to the compromised status of the DNA, determination of yield might vary between different quantification methods. While UV-Vis–based measurements will show high absorptions at A260, especially for DNA from samples with heavy fragmentation, fluorometric methodologies using dyes specific for dsDNA (e.g., Qubit) might by contrast show significantly lower DNA recovery. In addition, yield and PCR performance do not necessarily correlate; high yields of DNA as determined by either of the abovementioned methods might not show good PCR performance. This could be due to the quality of the FFPE sample regarding DNA fragmentation status and/or the efficiency of cross-link reversal prior to DNA extraction. DNA of a more fragmented status shows better PCR performance for short amplicons in PCR (<100bp) than DNA of higher molecular weight. However, highly fragmented DNA will not be suitable for PCR applications with amplicons larger than the size of the extracted DNA fragments. If de-crosslinking during DNA purification is insufficient, the extracted DNA will not be properly accessible despite sufficient integrity and poses a poor template for amplification of both small and large fragments in PCR. Thus, DNA yield measured by PCR may differ between large amplicon and short amplicon PCR systems and might also deviate from values obtained by UV-Vis–based or fluorometric quantification technologies.

It is recommended to use more than one quality control measure to evaluate DNA quality and quantity, focusing on which downstream application the DNA is intended to be used in. The EZ1&2 DNA FFPE UNG Kit and the EZ1&2 DNA FFPE Kit provide an optimized workflow for extraction of DNA for use in PCR, digital PCR, and NGS analysis allowing a selection between a fully automated procedure or automated bind, wash, and elute steps after manual processing (Figure 1).

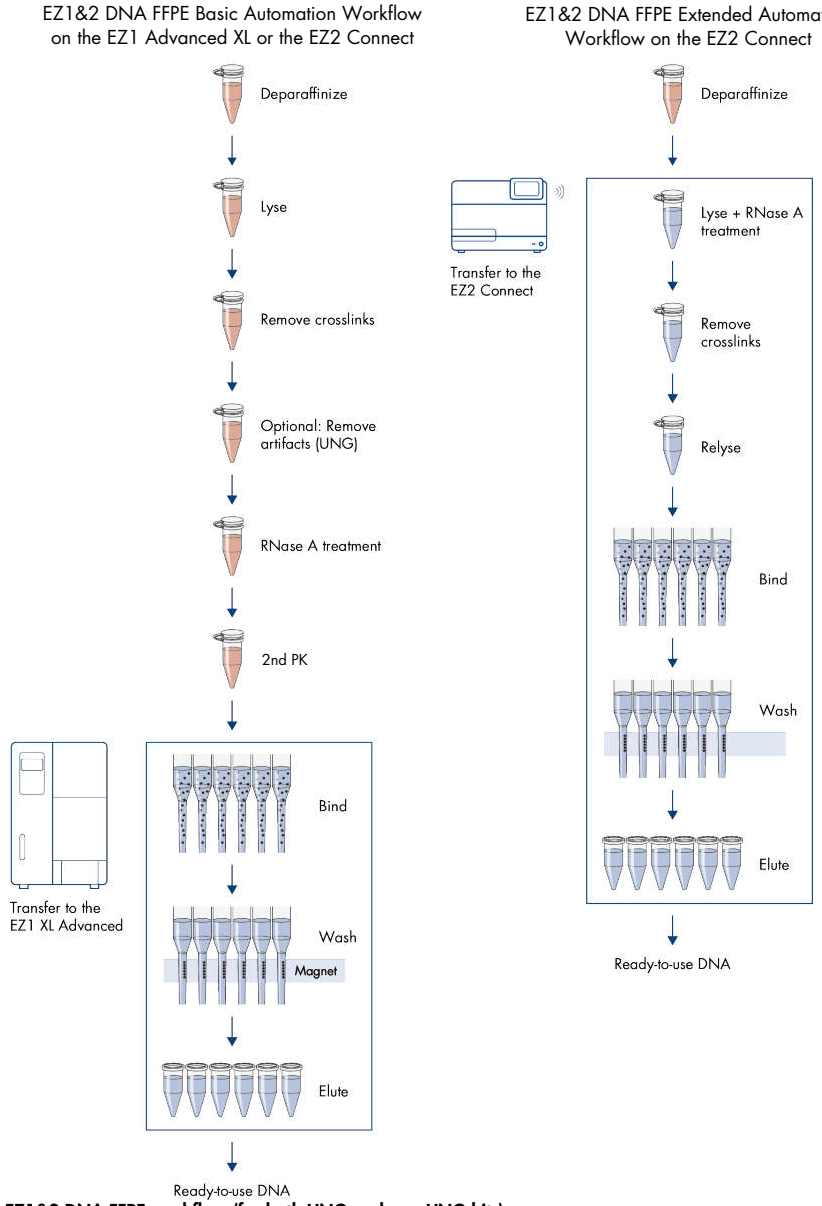


Figure 1. EZ1&2 DNA FFPE workflow (for both UNG and non-UNG kits)

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.

- EZ2 Connect instrument
- Shaker for microcentrifuge tubes capable of incubation at 90°C, for example, the Thermomixer® Comfort (cat. no. 5355 000.011) with appropriate block from Eppendorf® (**www.eppendorf.com**)
- Microcentrifuge with rotor for 2 ml tubes (up to 21,000 x *g*)
- Pipettors (2–1000 µl)
- Microcentrifuge Tubes (e.g., Safe-Lock Tubes [Eppendorf, cat. no. 0030 120.086 or 0030 120.094] or SafeSeal microcentrifuge tubes [Sarstedt®, cat no. 72.706 or 72.695.500])

Important Notes

Working with the EZ2 Connect Instrument

The main features of EZ2 Connect instrument 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 must 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.

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, labeled 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 the EZ2 Connect instrument is where the user equipped cartridge and tip racks (Figure 4).

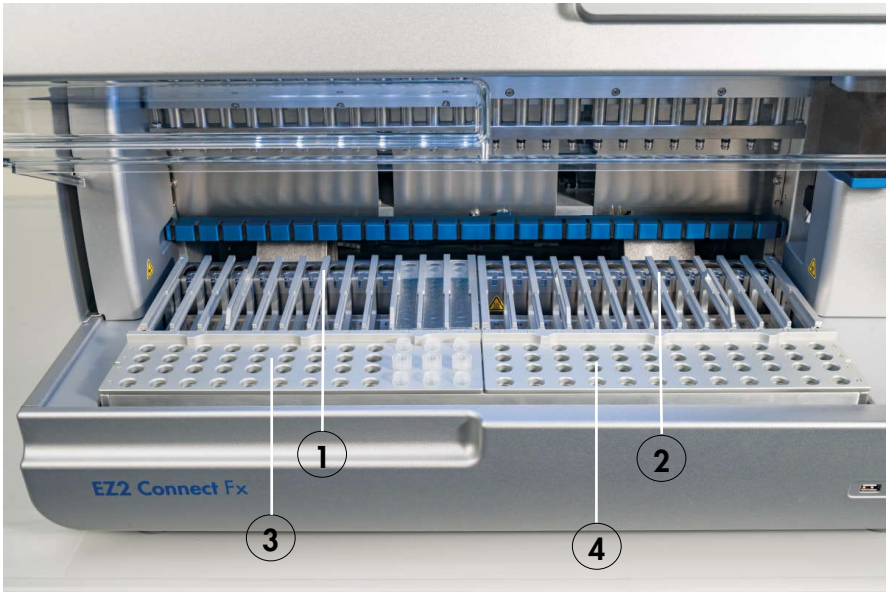


Figure 4. EZ2 Connect Worktable.

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 User Manual.*

Protocol: EZ1&2 DNA FFPE Kit

Important notes before starting

- Preheat a thermomixer at 80°C for use in step 2 and a second thermomixer at 56°C for use in step 4 if following the procedure for the "DNA FFPE bind, wash, elute" protocol.
- If Buffer FTB precipitates, heat at 30°C.
- Before loading reagent cartridges into the EZ2 Connect instrument, invert them 4 times to mix the magnetic particles and then tap to deposit the reagents at the bottom of the wells. Make sure that the magnetic particles are completely resuspended.

Procedure using the protocol for extended automation "DNA FFPE"

Note: The extended protocol automates all work steps after deparaffinization. UNG treatment cannot be included in the extended protocol. If UNG treatment is required, please refer to "Protocol: EZ1&2 DNA FFPE UNG Kit".

1. Place the FFPE sections in a 2 ml tube (supplied). Add 300 µl Paraffin Removal Solution, vortex vigorously for 10 s, and centrifuge briefly to bring the sample to the bottom of the tube.
2. Incubate for 2 min at 80°C.

   Optional: Vortex again after incubation, then briefly centrifuge the tube to remove drops from the inside of the lid and collect tissue at the bottom of the tube.
3. Turn on the EZ2 Connect instrument.
4. Tap "DNA" on the Applications panel and then select the "DNA FFPE Kit " and press **Next**.
5. Choose the "DNA FFPE" protocol 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 DNA FFPE reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 7.
10. Place the 2 ml tube containing the sample from step 2 into position 11 of the reagent cartridge (positions are labelled by engravings). Press **Next**.
11. Open instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.
12. Remove caps of all tubes and prepare the EZ2 Connect Tip Rack as follows (Figure 3):
    - Position A: 1.5 ml tube with 30 µl Buffer FTB and 25 µl Proteinase K
    - Position B: 1.5 ml tube with 100 µl RNase-free water and 4 µl RNase A
    - Position C: Tip holder with Filter Tip
    - Position D: 1.5 ml tube

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

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

Procedure using protocol for basic automation ("DNA FFPE bind, wash, elute")

Note: The basic protocol automates the bind, wash, and elution steps. Upfront work steps need to be carried out manually.

1. Place the FFPE sections at the bottom of a 1.5 ml or 2 ml microcentrifuge tube (not supplied). Add 300 µl Paraffin Removal Solution, vortex vigorously for 10 s, and centrifuge briefly to bring the sample to the bottom of the tube.
2. Incubate for 2 min at 80°C.

   Optional: Vortex again after incubation, then briefly centrifuge the tube to remove drops from the inside of the lid and collect tissue at the bottom of the tube.

   Note: After incubation, set the thermomixer to 90°C for incubation in step 5.
3. Add 25 µl Buffer FTB, 55 µl RNase-free water, and 20 µl Proteinase K. Mix thoroughly using, for example, a vortex instrument. Briefly centrifuge the tube to spin down any FFPE tissue that sticks to the tube wall or the cap.

   Note: A master mix that comprises the respective components may be prepared in advance.
4. Incubate for 1 h at 56°C with shaking at 1000 rpm.

   Note: After incubation, set the thermomixer set the thermomixer to 65°C for incubation in step 10.
5. Incubate for 1 h at 90°C.
6. Briefly centrifuge the tube to remove drops from the inside of the lid.
7. Carefully transfer the lower phase into a new microcentrifuge tube (not provided)
8. Add 150 µl RNase-free water, and then vortex.
9. Add 2 µl RNase A, vortex, and incubate for 2 min at room temperature.

   Optional: After incubation, briefly centrifuge the tube to remove drops from the inside of the lid.

10. Add 20 µl Proteinase K, vortex, and incubate for 15 min at 65°C and 450 rpm.

    Optional: After incubation, briefly centrifuge the tube to remove drops from the inside of the lid.

11. Transfer the sample into a 1.5 ml tube (provided) for use in step 20.

    Note: Each sample requires two 1.5 ml tubes: one for loading the sample onto the EZ2 Connect instrument and one to collect the DNA after purification. The worktable setup will guide you.

12. Turn on the EZ2 Connect instrument.
13. Tap "DNA" on the Applications panel and then select the "DNA FFPE Kit " and press **Next**.
14. Choose the "DNA FFPE bind, wash, elute" protocol and press **Next**.
15. Choose the elution volume and press **Next**.
16. Select positions on the work deck according to the number of samples to be processed and press **Next**.
17. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
18. Load the EZ1&2 DNA FFPE reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 16.
19. Open instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.
20. Remove caps of all tubes and prepare the EZ2 Connect Tip Rack as follows (Figure 3):
    - Position A: 1.5 ml tube with sample from step 11
    - Position B: empty
    - Position C: Tip holder with Filter Tip
    - Position D: 1.5 ml tube

    Press **Next**.
21. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the protocol following instructions on the instrument display.
22. The display will show "Protocol finished" when the run is completed. Select **Finish**.

23. Open the instrument hood. Remove the elution tube containing purified DNA from position D of the EZ2 Connect Tip Rack. Discard the used EZ2 cartridge including the liquid waste.

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

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

Protocol: EZ1&2 DNA FFPE UNG Kit

Important notes before starting

- Preheat a thermomixer at 80°C for use in step 2 and a second thermomixer at 56°C for use in step 4. If available, preheat additional thermomixers to 90°C and 50°C respectively, or else follow the instructions as described.
- If Buffer FTB precipitates, heat to 30°C before using.
- Before loading reagent cartridges into the EZ2 Connect instrument, invert them 4 times to mix the magnetic particles and then tap to deposit the reagents at the bottom of the wells. Make sure that the magnetic particles are completely resuspended.

Procedure for the EZ1&2 DNA FFPE UNG kit using the "DNA FFPE bind, wash, elute" protocol

1. Place the FFPE sections in a 1.5 ml or 2 ml microcentrifuge tube (not supplied). Add 300 µl Paraffin Removal Solution, vortex vigorously for 10 s, and centrifuge briefly to bring the sample to the bottom of the tube.
2. Incubate for 2 min at 80°C.

   Optional: Vortex again after incubation, then briefly centrifuge the tube to remove drops from the inside of the lid and collect tissue at the bottom of the tube.

   Note: After incubation, set the thermomixer to 90°C for incubation in step 5.
3. Add 25 µl Buffer FTB, 55 µl RNase-Free Water, and 20 µl Proteinase K. Mix thoroughly using, for example, a vortex instrument. Briefly centrifuge the tube to spin down any FFPE tissue that sticks to the tube wall or the cap.

   Note: A master mix that comprises the respective components with a total volume of 100 µl per sample may be prepared in advance
4. Incubate for 1 h at 56°C and 1000 rpm.

   Note: After incubation, set the thermomixer to 50°C for incubation in step 7.

5. Incubate for 1 h at 90°C.
6. Briefly centrifuge the tube to remove drops from the inside of the lid.
7. Carefully transfer the lower phase into a new microcentrifuge tube (not provided)
8. Add 115 µl RNase-Free Water and 35 µl UNG. Vortex and incubate at 50°C for 5 min without shaking.

   Optional: After incubation, briefly centrifuge the tube to remove drops from inside the lid.

   Note: After incubation, set the thermomixer to 65°C for incubation in step 10.
9. Add 2 µl RNase A, vortex, and incubate for 2 min at room temperature on the bench.

   Optional: After incubation, briefly centrifuge the tube to remove drops from inside the lid.
10. Add 20 µl Proteinase K, vortex, and incubate for 15 min at 65°C and 450 rpm.
11. **Optional**: After incubation, briefly centrifuge the tube to remove drops from inside the lid.
12. Transfer the sample into a 1.5 ml tube (provided) for use in step 21

    Note: Each sample requires two 1.5 ml tubes: one for loading the sample onto the EZ2 Connect instrument and one to collect the DNA after purification. The worktable setup will guide you.
13. Turn on the EZ2 Connect instrument.
14. Tap "DNA" on the Applications panel and then select the "DNA FFPE Kit " and press **Next**.
15. Choose the "DNA FFPE bind, wash, elute" protocol and press **Next**.
16. Choose the elution volume and press **Next**.
17. Select positions on the work deck according to the number of samples to be processed and press **Next**.
18. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
19. Load the EZ1&2 DNA FFPE reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 16.
20. Open instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.

21. Remove caps of all tubes and prepare the EZ2 Connect Tip Rack as follows (Figure 3):
    - Position A: 1.5 ml tube with sample from step 12
    - Position B: empty
    - Position C: Tip holder with Filter Tip
    - Position D: 1.5 ml tube

    Press **Next**.
22. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the protocol following instructions on the instrument display.
23. The display will show "Protocol finished" when the run is completed. Select **Finish**.
24. Open the instrument hood. Remove the elution tube containing purified DNA from position D of the EZ2 Connect Tip Rack. Discard the used EZ2 cartridge including the liquid waste.

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

    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
Statement of the problem	
a) Poor quality of starting material	Samples that were fixed for over 20 hours or stored for very long periods of time may contain very little usable nucleic acids. Sections that were mounted on microscope slides may yield very little usable nucleic acids due to prolonged exposure to air.
b) Insufficient reagent aspirated	After inverting the reagent cartridges to resuspend the magnetic particles, make sure to tap the cartridges to deposit the reagents at the bottom of the wells.
b) Magnetic particles not completely resuspended	Make sure to resuspend the magnetic particles thoroughly before loading the reagent cartridges into the holder.
Inefficient removal of deaminated cytosine	
a) Too much starting material	Since the EZ1&2 DNA FFPE UNG Kit is based on an enzymatic digestion, too much starting material will lead to inefficiency. Reduce the amount of starting material.
b) UNG reaction mixture prepared incorrectly	Be sure to properly prepare the reaction mix by precise addition of all components and transfer of the aqueous phase in steps 3 and 7.
General handling	
a) Error message in instrument display	Refer to the user manual supplied with your EZ2 Connect instrument.
c) Wrong Q-Card ID entered	If the wrong ID was entered instead of the Q-Card ID, the EZ2 Connect 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.

Ordering Information

Product	Contents	Cat. no.
EZ2 Connect	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
EZ1&2 DNA FFPE Kit (48)	For 48 preps: Uracil-N-Glycosylase, Paraffin Removal Solution, EZ1&2 DNA FFPE cartridge, Filter Tips and Holders, Tubes, Proteinase K, RNase A, RNase-Free Water, and Buffers	954404
EZ1&2 DNA FFPE UNG Kit (48)	For 48 preps: Paraffin Removal Solution, EZ1&2 DNA FFPE cartridge, Filter Tips and Holders, Tubes, Proteinase K, RNase A, RNase-Free Water, and Buffers	954414
Uracil-N-Glycosylase (2 x 1 ml)	For use with the EZ1&2 DNA FFPE Kit, 50 preps	19160
Accessories and reagents		
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
RNase A (17,500 U)	2.5 ml (100 mg/ml; 7000 units/ml, solution)	19101

Product	Contents	Cat. no.
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
04/2022	Initial revision

Notes

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