February 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 the EZ1® Advanced XL instrument

Sample to Insight

QIAGEN

Contents

Kit Contents 3

Shipping and Storage 4

Intended Use 5

Safety Information 5

Quality Control 6

Introduction 7

- Principle and procedure 8
- Automation 8
- Starting material 9
- DNA quality and yield 11

Equipment and Reagents to Be Supplied by User 13

Important Notes 14

- Working with the EZ1 Advanced XL instrument 14
- Workflow of the EZ1 Advanced XL DNA FFPE operation 19

Protocol: EZ1&2 DNA FFPE UNG Kit 20

Protocol: EZ1&2 DNA FFPE Kit 22

Troubleshooting Guide 24

Ordering Information 25

Document Revision History 27

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	1 x 50	1 x 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 EZ1 Advanced XL instruments and 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 EZ1 Advanced XL instruments 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 Kit and the EZ1&2 DNA FFPE Kit are tested against predetermined specifications to ensure consistent product quality.

Introduction

This handbook describes processing of the EZ1&2 DNA FFPE and EZ1&2 DNA FFPE UNG Kits with EZ1 instruments. For usage of the EZ1&2 DNA FFPE and EZ1&2 DNA FFPE UNG Kits with EZ2 instruments, please refer to the handbook (**www.qiagen.com/HB-3015-001**) and quick-start protocol (**www.qiagen.com/HB-3017-001**).

Exposure to formalin result 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 in order 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

The EZ1&2 DNA FFPE and EZ1&2 DNA FFPE UNG procedures remove paraffin using non-hazardous Paraffin Removal Solution 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). After RNase A digestion and 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 60 or 100 µl 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 EZ1 Advanced XL instrument. Digestion with Proteinase K, cross-link removal and RNase A digestion are carried out manually. DNA binding, washing and elution steps are conducted on the EZ1 Advanced XL instrument.

Automation of the complete workflow can be done with the EZ2 Connect instrument. For more information about the EZ2 Connect instrument, please refer to the handbook (**www.qiagen.com/HB-3015-001**) and quick-start protocol (**www.qiagen.com/HB-3017-001**).

Starting material

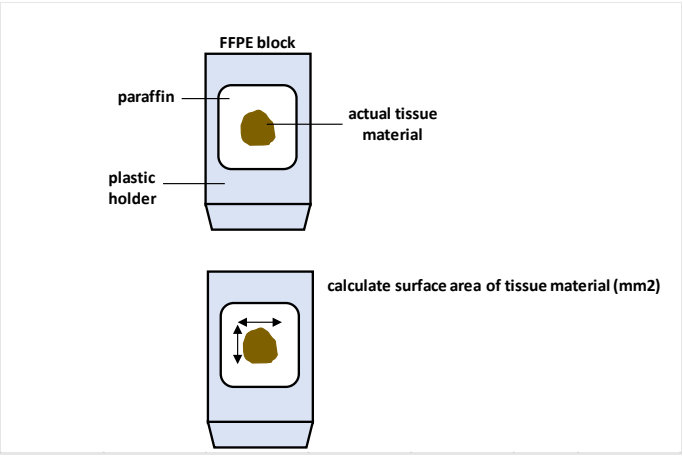
Typical formalin-fixation and paraffin-embedding procedures result in significant fragmentation of nucleic acids. To limit the extent of nucleic acid fragmentation, please use following guidelines:

- 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 with regard to DNA fragmentation status and/or the efficiency of cross-link reversal prior to DNA extraction. DNA of a more fragmented status shows far 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 (Figure 1).

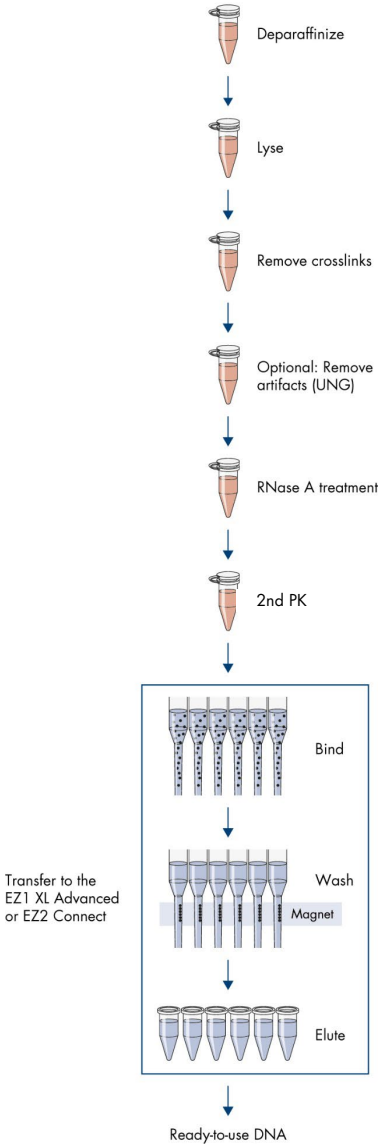


Figure 1. EZ1&2 DNA FFPE workflow (for both UNG and non-UNG kits) on the EZ1 Advanced XL

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.

- EZ1 Advanced XL instrument
- EZ1 Advanced XL DNA FFPE Card (cat. no. 9026946)
- 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 EZ1 Advanced XL instrument

The main features of the EZ1 Advanced XL instrument include the following:

- Purification of high-quality nucleic acids from 1–14 samples per run
- Small footprint to save laboratory space
- Preprogrammed EZ1 Advanced XL Cards containing ready-to-use protocols for nucleic acid purification
- Prefilled, sealed reagent cartridges for easy, safe, and fast setup of the EZ1 Advanced XL instrument
- Bar code reading and sample tracking
- Kit data tracking with the Q-Card provided in the kit
- UV lamp to help eliminate sample carryover from run-to-run and to allow decontamination of the worktable surfaces

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

EZ1 Advanced XL Cards

Protocols for nucleic acid purification are stored on a preprogrammed EZ1 Cards (integrated circuit cards). The user simply inserts an EZ1 Advanced XL Card into the EZ1 Advanced XL instrument, and the instrument is then ready to run a protocol (Figure 2 and Figure 3). The availability of various protocols increases the flexibility of EZ1 instruments.

Figure 2. Ease of protocol setup using EZ1 Advanced XL Cards. Inserting an EZ1 Card, containing a protocol, into the EZ1 Advanced XL instrument.

The instrument should only be switched on after an EZ1 Card is inserted. Make sure that the EZ1 Card is completely inserted! Otherwise, essential instrument data could be lost, leading to a memory error. EZ1 Cards should not be exchanged while the instrument is switched on.

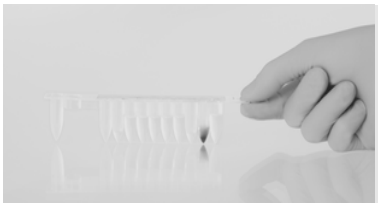
Figure 3. EZ1 Card completely inserted into EZ1 Card slot.

Reagent cartridges

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

A



B

Figure 4. Ease of instrument setup using reagent cartridges. A A sealed, prefilled reagent cartridge of the EZ1 ccfDNA Mini or Midi Kit. **B** Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded.

Worktable

The worktable of the EZ1 Advanced XL instrument is where the user loads samples and the components of the EZ1&2 DNA FFPE Kits (Figure 5).

Details on worktable setup are provided in the protocol in this handbook, and are also displayed in the vacuum fluorescent display (VFD) of the EZ1 Advanced XL control panel when

the user starts the worktable setup. The display also shows protocol status during the automated purification procedure.

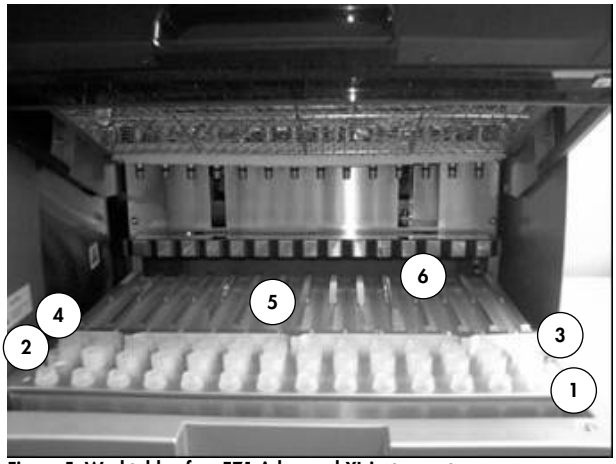


Figure 5. Worktable of an EZ1 Advanced XL instrument.

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

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

3 Third row: For the EZ1&2 DNA FFPE or EZ1&2 DNA FFPE UNG Kit protocol, this row is empty.

4 Fourth row: Elution tubes (ET) (1.5 ml) containing the sample prepared with the EZ1&2 DNA FFPE or EZ1&2 DNA FFPE UNG workflow.

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

6 The heating block is empty for the EZ1&2 DNA FFPE or EZ1&2 DNA FFPE UNG Kit protocol.

Data tracking with the EZ1 Advanced XL

The EZ1 Advanced XL enables complete tracking of a variety of data for increased process control and reliability. The EZ1 Kit lot number and expiration date are entered at the start of the protocol using the Q-Card bar code. A user ID and the Q-Card bar code can be entered manually using the keypad or by scanning bar codes using the handheld bar code reader. Sample and assay information can also be optionally entered at the start of the protocol. At the end of the protocol run, a report file is automatically generated. The EZ1 Advanced XL can store up to 10 report files, and the data can be transferred to a PC or directly printed on a printer (see “Equipment and Reagents to Be Supplied by User”, page 13).

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. In report files, the 14 pipetting channels of the EZ1 Advanced XL are named, from left to right, channels 1–14.

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

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

Workflow of the EZ1 Advanced XL DNA FFPE operation

Insert EZ1 Card into the EZ1 Card slot

↓

Switch on the EZ1 instrument

↓

Follow onscreen messages for data tracking

↓

Follow onscreen messages for worktable setup

↓

Start the protocol

↓

Collect purified nucleic acids

↓

UV decontamination

Protocol: 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 preheated 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 EZ1 Advanced XL instrument, invert them 3 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

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), and 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 9.
8. 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.
9. 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 inside the lid.
10. Transfer the sample into a 1.5 ml sample tube (provided) for use in step 14.

    Note: Each sample requires two 1.5 ml tubes: one for loading the sample onto the EZ1 instrument and one to collect the DNA after purification. The worktable setup in step 14 will guide you.
11. Insert the EZ1 Advanced XL DNA FFPE Card completely into the EZ1 Advanced XL instrument; switch on the instrument.
12. Press **START** to start the worktable setup of the EZ1 DNA FFPE protocol.
13. Choose the elution volume: press **1** to elute in 60 µl or **2** to elute in 100 µl.
14. Open the instrument door. Follow the onscreen instructions for worktable setup and data tracking. Close the instrument door; press **START** to start the protocol.
15. The display will show **Protocol finished** when finished. Press **ESC**.
16. Open the instrument door. Remove the elution tubes containing the purified DNA from the first row of the rack. Discard the sample preparation waste*. Press **ENT**. The report file is transferred automatically.

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

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

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 available preheated additional thermomixers to 90°C and 65°C respectively, or else follow the instructions as described.
- If Buffer FTB precipitates, heat at 30°C.
- Before loading reagent cartridges into the EZ1 Advanced XL instrument, invert them 3 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

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 65°C for incubation in step 9.
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), and add 150 µl RNase-Free Water, and then vortex.

   Optional: After incubation, briefly centrifuge the tube to remove drops from inside the lid.
8. 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.
9. 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 inside the lid.
10. Transfer the sample into a 1.5 ml sample tube (provided) for use in step 14.

    Note: Each sample requires two 1.5 ml tubes: one for loading the sample onto the EZ1 instrument and one to collect the DNA after purification. The worktable setup in step 14 will guide you.
11. Insert the EZ1 Advanced XL DNA FFPE Card completely into the EZ1 Advanced XL instrument; switch on the instrument.
12. Press **START** to start the worktable setup of the EZ1 DNA FFPE protocol.
13. Choose the elution volume: press **1** to elute in 60 µl or **2** to elute in 100 µl.
14. Open the instrument door. Follow the onscreen instructions for worktable setup and data tracking. Close the instrument door; press **START** to start the protocol.
15. The display will show **Protocol finished** when finished. Press **ESC**.
16. Open the instrument door. Remove the elution tubes containing the purified DNA from the first row of the rack. Discard the sample preparation waste*. Press **ENT**. The report file is transferred automatically.

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

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

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page 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 EZ1 Advanced XL instrument.
b) Report file not printed	Check whether the printer is connected to the EZ1 Advanced XL via the "PC/Printer" serial port. Check whether the serial port is set for use with a printer.
c) Wrong Q-Card ID entered	If the wrong ID was entered instead of the Q-Card ID, the EZ1 Advanced XL will not accept the ID and will prompt for the Q-Card ID until the correct ID is entered. Press **STOP** twice to go to the main menu.

Ordering Information

Product	Contents	Cat. no.
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
EZ1 Advanced XL DNA FFPE card	Preprogrammed card for EZ1 Advanced XL DNA FFPE protocol	9026946
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 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

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

Date	Changes
04/2021	Initial revision
02/2022	Update the component list in the Kit Contents section. Added references to the handbook and quick-start protocol for processing on EZ2 Connect.

Notes

Limited License Agreement for EZ1&2 DNA FFPE and EZ1&2 DNA FFPE UNG Kits

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
2. Other than expressly stated licenses, QIAGEN makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.
3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see **www.qiagen.com**.

HB-2867-002 02/2022