April 2021

Quick-Start Protocol

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

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). This protocol version describes usage of EZ1&2 DNA FFPE Kit on the EZ1 Advanced XL instrument. For more information about the EZ2 Connect instrument please visit **www.qiagen.com/EZ2Connect-updates**. UNG is shipped on dry ice and, upon receipt, should be stored 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.

Proteinase K is stable for at least 1 year after delivery when stored at room temperature. For storage longer than 1 year, Proteinase K should be stored at 2–8°C.
All other components of the EZ1&2 DNA FFPE kit should be stored dry at room temperature (15–25°C). Under these conditions, they are stable until the expiration date on the kit box.

Further information

- *EZ1&2 DNA FFPE Kit and EZ1&2 DNA FFPE UNG Kit Handbook*: **www.qiagen.com/HB-2867**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

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 a precipitate has formed in Buffer FTB, dissolve by incubating at 30°C.
- Process FFPE tissue sections of 5–10 µm thickness, totaling up to 4 mm^3 of tissue can be proceeded. If it is not possible to calculate the exact amount of tissue, use no more than 2 sections of 5–10 µm thickness each.
- 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 at the bottom of a 1.5 ml or 2 ml microcentrifuge tube (not provided). Add 300 µl of 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 by vortexing. 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 and 1000 rpm.

   Note: After incubation, set the thermomixer to 50°C for incubation in step 7a if using the EZ1&2 DNA FFPE UNG Kit. If using the EZ1&2 DNA FFPE Kit, 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).

   7a. **EZ1&2 DNA FFPE UNG Kit**: Add 115 µl RNase-free water and 35 µl UNG, vortex, and incubate for 5 min at 50°C.

   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.

   7b. **EZ1&2 DNA FFPE Kit**: Add 150 µl RNase-free water, and then vortex.

8. 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.

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 the inside of the lid.

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

    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 mentioned in step 12 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. When the protocol ends, the display shows **Protocol 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.

Document Revision History

Date	Changes
03/2021	Initial release
04/2021	Changed the kit name.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

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