Quick-Start Protocol March 2022

EZ1&2™ DNA FFPE Kit and EZ1&2 DNA FFPE UNG Kit

For use with EZ2® Connect instruments

For usage of EZ1&2 DNA FFPE with EZ1 instruments, refer to respective handbook (**www.qiagen.com/HB-2867-002**) and quick-start protocol (**www.qiagen.com/HB-2852-001**).

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). UNG is shipped on dry ice and, upon receipt, should be stored at –30 to –15°C. Under these conditions, UNG is stable until the expiration day printed on the tube label. Proteinase K is stable for at least 1 year after delivery when stored at room temperature. For longer storage, 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 Handbook for use with EZ2 Connect*: **www.qiagen.com/HB-3015**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Preheat a thermomixer to respective temperatures to save time
- If Buffer FTB precipitates, heat at 30°C.
- FFPE tissue sections of 5–10 µm thickness, totaling up to 4 mm^3 of tissue can be processed.

Procedure for automated extraction without UNG step

1. Place the FFPE sections at the bottom of a 2 ml tube (provided). Add 300 µl Paraffin Removal Solution, vortex vigorously for 10 s.
2. Incubate for 2 min at 80°C and vortex afterwards.
3. Switch 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 elution volume and press **Next**.
7. Select positions on the work deck and press **Next**.
8. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
9. Gently invert reagent cartridges 4 times to mix the magnetic particles. And then, tap the cartridges to deposit the reagents at the bottom of their wells.
10. Load the EZ1&2 DNA FFPE reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 7.
11. Place the 2 ml tube containing the sample from step 2 into position 11 of the reagent cartridge.
12. Open EZ2 Connect instrument hood and Load the EZ2 Connect Cartridge Rack.
13. Remove caps of all tubes and prepare the EZ2 Connect Tip Rack as follows:
    - 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 empty elution tube

    Press **Next**.
14. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run.
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 DNA from position D of the EZ2 Connect Tip Rack. Discard the used cartridge.
17. Perform regular maintenance after each run. Press **Finish** to return to the home screen.

Procedure for manual pre-treatment and optional UNG step

1. Place the FFPE sections at the bottom of 1.5 ml or 2 ml microcentrifuge tube (not provided). Add 300 µl Paraffin Removal Solution, vortex vigorously for 10 s.
2. Incubate for 2 min at 80°C and vortex.
3. Add 25 µl Buffer FTB, 55 µl RNase-free water, and 20 µl Proteinase K. Mix thoroughly.
4. Incubate for 1 h at 56°C with shaking at 1,000 rpm.

   Note: After incubation, set the thermomixer to 50°C for incubation in step 6a if performing the UNG step. Otherwise, set the thermomixer to 65°C for incubation in step 8.
5. Incubate for 1 h at 90°C.
6. Carefully transfer the lower phase into a new microcentrifuge tube (not provided)

   6a. 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.

   Note: After incubation, set the thermomixer to 65°C for incubation in step 8.

   6b. EZ1&2 DNA FFPE Kit: Add 150 µl RNase-free water and vortex.
7. Add 2 µl RNase A, vortex, and incubate for 2 min at room temperature.
8. Add 20 µl Proteinase K, vortex, and incubate for 15 min at 65°C and 450 rpm.
9. Transfer the sample into a 1.5 ml tube (provided) for use in step 19.
10. Switch on the EZ2 Connect instrument.
11. Tap "DNA" on the Applications panel and then select the "DNA FFPE Kit" and press **Next**.
12. Choose the "DNA FFPE bind wash elute" protocol and press **Next**.
13. Choose elution volume, and press **Next**.
14. Select positions on the work deck and press **Next**.
15. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**

16. Gently invert reagent cartridges 4 times to mix the magnetic particles. And then, tap the cartridges to deposit the reagents at the bottom of their wells.
17. Load the reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack.
18. Open EZ2 Connect instrument hood and Load the EZ2 Connect Cartridge Rack.
19. Remove caps of all tubes and prepare the EZ2 Connect Tip Rack as follows:
    - Position A: 1.5 ml tube with sample from step 9
    - Position B: Empty
    - Position C: Tip holder with Filter Tip
    - Position D: 1.5 ml empty elution tube

    Press **Next**.
20. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run.
21. The display will show "Protocol finished" when the run is completed. Select **Finish**.
22. Open the instrument hood. Remove the elution tube containing purified DNA from position D of the EZ2 Connect Tip Rack. Discard the used cartridge.
23. Perform regular maintenance after each run. Press **Finish** to return to the home screen.

Document Revision History

Date	Changes
03/2022	Initial release

Scan QR code for handbook.

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