Quick-Start Protocol

July 2023

EZ2® AllPrep® DNA/RNA FFPE Kit

For use with EZ2 Connect instruments

The EZ2 AllPrep DNA/RNA FFPE Kit (cat. no. 954734) is shipped at room temperature (15–25°C). Upon receipt, store the DNase I at 2–8°C. Store all other kit components dry at room temperature.

Further information

- *EZ2 AllPrep DNA/RNA FFPE Kit Handbook*: **qiagen.com/HB-2946**
- Safety Data Sheets: **qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Set a thermal mixer to 56°C.
- Before first use, resuspend the DNase I with 550 µL of the supplied RNase-free water. The solution can be stored at 2–8°C for up to 4 weeks or should be aliquoted and stored at –30°C to –15°C for extended time periods while avoiding freeze–thaw cycles
- Before starting the procedure, check whether precipitate has formed in Buffer ATL. If necessary, dissolve by heating to 70°C with gentle agitation.

Procedure

1. Trim excess paraffin off the sample block using a scalpel.
2. Cut the paraffin-embedded sample into sections of 10 µm thickness. If the sample surface has been exposed to air, discard the first 2–3 sections. The sample volume should not exceed 2 mm^3.
3. Immediately place the sections into a 2 mL sample tube (provided).
4. Add 320 µL Paraffin Removal Solution (PRS) and close the cap.
5. Vortex vigorously for 10 s, and centrifuge briefly to bring the sample to the bottom of the tube.
6. Incubate at 56°C for 3 min and centrifuge at 20,000 x *g* for 2 min.
7. Carefully remove the supernatant by pipetting without disturbing the pellet.
8. Resuspend the pellet by adding 150 µL Buffer PKD and flick the tube to loosen the pellet. Add 10 µL Proteinase K and mix by vortexing briefly.
9. Place the tubes in a thermal mixer and incubate at 56°C for 15 min at 500 rpm. Incubate on ice for 3 min. Set a thermal mixer to 80°C.
10. Centrifuge for 15 min at 20,000 x *g*. Carefully transfer the supernatant, without disturbing the pellet, to a new 1.5 mL microcentrifuge tube for RNA preparation (step 12).
11. Keep the pellet for DNA preparation (step 16).

RNA preparation

12. Incubate the RNA-containing supernatant from step 10 at 80°C for 15 min. Incubate on ice for 3 min.
13. Add 16 µL DNase booster buffer and mix by vortexing.
14. Add 10 µL DNase I, mix gently by inverting the tube, and spin down.
15. Incubate at room temperature for 15 min.

DNA preparation

16. Resuspend the DNA-containing pellet from step 11 in 180 µL Buffer ATL, add 40 µL Proteinase K, and mix by vortexing briefly. Spin down to collect the sample at the bottom of the tube.
17. Overlay this suspension carefully with 200 µL PRS.

EZ2 preparation

18. Turn on the EZ2 Connect instrument.

    Tap **RNA** on the Applications panel and select the **AllPrep DNA/RNA FFPE Kit** and press **Next**. Follow onscreen instructions for selection of protocol, parameter definition, sample position selection, sample IDs, and worktable setup. Ensure the heating block of the EZ2 instrument is at room temperature.
19. Load the EZ2 AllPrep DNA/RNA FFPE reagent cartridges into the EZ2 Connect Cartridge Rack.
20. Transfer the RNA lysate from step 15 into position 12 of the EZ2 AllPrep DNA/RNA FFPE cartridge.
21. Place the 2 mL sample tube containing the DNA pellet from step 17 into position 11 of the reagent cartridge (positions are labelled with engravings on the EZ2 Connect Cartridge Rack).
22. Open instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.

23. Remove caps of all tubes and prepare the EZ2 Connect Tip Rack as described below (positions are labelled with engravings on the EZ2 Connect Tip Rack):
    - Position A: Tip holder with Filter Tip
    - Position B: Tip holder with Filter Tip
    - Position C: new 1.5 mL elution tube
    - Position D: new 1.5 mL elution tube
24. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.
25. The display will show "Protocol finished" when the run is completed. Select **Finish**.
26. Open the instrument hood. Remove the elution tube containing the purified DNA from position C and the elution tube containing the purified RNA 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.
27. Perform regular maintenance after each run. Press **Finish** to return to the Home Screen.

Document Revision History

Date	Changes
October 2021	Initial release
July 2023	Updated "Notes before starting". Improved the description of steps in "RNA preparation", "DNA preparation", and "EZ2 preparation".

Scan QR code for handbook.

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