Quick-Start Protocol May 2024

EZ2® PowerFecal® Pro DNA/RNA Kit

The EZ2 PowerFecal Pro DNA/RNA Kit is shipped at room temperature (15–25°C). Upon receipt, Solution CD2 should be stored at 2–8°C. All other reagents and kit components should be stored at room temperature.

Further information

- *EZ2 PowerFecal Pro DNA/RNA Kit Handbook*
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Equipment & Reagents Required

- RNA-only protocol: RNase-free DNase Set (50) (cat.no. 79254), not provided
- DNA-only protocol: RNase A (17,500 U) (cat.no. 19101), not provided
- Phenol–chloroform–isoamyl alcohol (25:24:1) pH 6.5-8, not provided
- Microcentrifuge (with rotor for 2 mL tubes)
- Equipment for sample disruption and homogenization, one of the following:
    - Vortex Genie 2 and Vortex Adapter for 24 (1.5–2 mL) tubes (cat. no. 13000-V1-24)
    - TissueLyser III (cat. no. 9003240) with adapter sets for use with the PowerBead Pro Tubes (TissueLyser Adapter Set 2 x 24 [cat. no. 69982] or 2 mL Tube Holder [cat. no. 11993], in conjunction with Plate Adapter Set, cat.no. 11990)

Notes before starting

- RNA-only protocol: Dissolve lyophilized DNase I (1500 Kunitz units) in 550 µL of RNase-free water. Mix gently by inverting. Do not vortex. For long-term storage store single-use aliquots at –15°C to –25°C. Thawed aliquots can be stored at 2–8° for 6 weeks.

Procedure

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.

2. Add up to 100 mg of stool, 700 µL of Solution CD1, 100 µL Solution CD2, and 100 µL phenol–chloroform–isoamyl alcohol (25:24:1, pH 6.5–8.0) to the PowerBead Pro Tube (in that order), and vortex briefly to mix.
3. Mechanically disrupt the samples using one of the following methods:
   a) Use a Vortex Genie 2. Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 24 (1.5–2 mL) tubes. Orient the tube caps to point toward the center of the vortex adapter. Vortex at maximum speed for 10 min.
   b) Use a TissueLyser III. Place the PowerBead Pro Tube into the TissueLyser Adapter Set 2 x 24 or 2 mL Tube Holder and Plate Adapter Set. Fasten the adapter into the instrument and shake for 5 min at 25 Hz speed. Re-orient the adapter so that the side that was closest to the machine body is now furthest from it. Shake again for 5 min at 25 Hz speed.
4. Centrifuge the PowerBead Pro Tube at 18,000 x *g* for 5 min.
5. Load reagent cartridges into the cartridge rack (invert cartridge 4 times to mix beads).

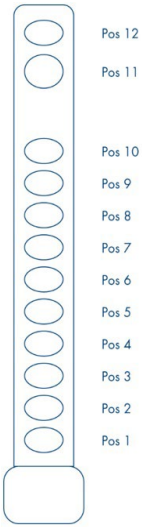

Figure 1. EZ2 PowerFecal Pro cartridge.

6. RNA-only protocol: Transfer 40 µL Buffer RDD and 10 µL resuspended DNase I into position 12 of the EZ2 PowerFecal Pro cartridge.
7. Remove caps of all tubes and prepare the Tip Rack as follows (see Figure 2):

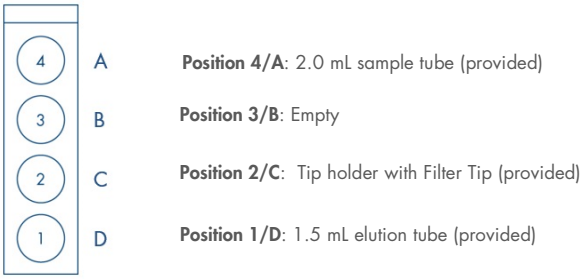

Figure 2. Tip Rack.

8. Transfer 600 µL sample supernatant after centrifugation in step 4 into a 2 mL sample tube in position 4/A. For DNA-only protocol, pipette 4µL RNase A into the sample supernatant (mixing is not required).

Procedure on the EZ2 Connect

9. Turn on the EZ2 Connect instrument.
10. For all protocols, tap **Microbe** on the Applications panel, then select the **EZ2 PowerFecal Pro Kit,** and press **Next.** Follow onscreen instructions for selection of protocol (DNA-only, RNA-only, and total nucleic acid), parameter definition, sample position selection, sample IDs, and worktable setup.
11. Open the instrument door. Load the cartridge rack into the instrument.
12. Place the tip rack into the instrument.
13. Close the instrument door; press **Start** to initiate the EZ2 PowerFecal Pro protocol.
14. The display will show “Protocol finished” when the run is completed. Select **Finish**.

Open the instrument hood. Remove the elution tubes containing the purified nucleic acid from position 1/D of the tip rack. Discard the sample preparation waste (in tubes in positions 2/C and 4/A) (see Figure 2)

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

15. Perform regular maintenance after each run. Press **Finish** to return to the Home Screen.

Document Revision History

Date	Changes
05/2024	Initial release.

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