February 2022

Quick-Start Protocol

AllPrep® PowerFecal® Pro DNA/RNA Kit

Solution CD2 should be stored at 2–8°C upon arrival. All other reagents and kit components should be stored at room temperature (15–25°C) until the expiry date printed on the box label.

AllPrep PowerFecal Pro DNA/RNA Kit is for the simultaneous isolation of microbial genomic DNA and total RNA from stool samples in two separate eluates.

Further information

- *AllPrep PowerFecal Pro DNA/RNA Kit Handbook*: **www.qiagen.com/HB-2851**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Phenol-chloroform-isoamyl alcohol (25:24:1, pH 6.5-8.0) is required for this protocol.
- Ensure that the PowerBead Pro Tubes rotate freely in the centrifuge without rubbing.
- Perform all centrifugation steps at room temperature.
- Refer to the *AllPrep PowerFecal Pro DNA/RNA Kit Handbook* for optimal homogenization method in step 3.

Procedure

Sample Pretreatment

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.
2. Add 50–100 mg of stool, 650 µl of Solution CD1 and 100 µl phenol–chloroform–isoamyl alcohol (25:24:1, pH 6.5–8.0) to the PowerBead Pro Tube and vortex briefly to mix.
3. Secure the PowerBead Pro Tube horizontally on a vortex adapter for 1.5–2 ml tubes (cat. no. 13000-V1-24). Orient tube caps to point toward the center of the Vortex Adapter. Vortex at maximum speed for 10 min.

   Note: If using the vortex adapter for more than 12 preps simultaneously, increase the vortex time by 5–10 min.

   For more information about other bead beating methods, see the "Protocol: Detailed" section of *AllPrep PowerFecal Pro DNA/RNA Kit Handbook*.
4. Centrifuge the PowerBead Pro Tube at 15,000 x *g* for 1 min. Transfer the supernatant to a clean 2 ml microcentrifuge tube (provided).

   Note: Expect a supernatant volume of 500–600 µl. The supernatant may still contain some stool particles.
5. Add 200 µl Solution CD2 and vortex for 5 s. Centrifuge at 15,000 x *g* for 1 min at room temperature.
6. Avoiding the pellet, transfer 300 µl of supernatant to a clean 2 ml microcentrifuge tube (provided).

   Note: It is feasible to use higher supernatant volumes, please refer to the HB for detailed information.
7. Add 300 µl of Solution CD3. Vortex briefly to mix.

DNA binding

8. Load 600 µl supernatant-CD3 mix into a MB DNA Spin Column (white) and centrifuge at 15,000 x *g* for 1 min collecting the flow-through in a 2 ml tube for RNA purification, then place the spin column in a new 2 ml collection tube.

RNA binding

9. Add 300 µl 96–100% ethanol to the flow-through from step 8 and mix by pipetting up and down.

   Note: If you used a higher volume of the supernatant in step 6, you would have to adjust the binding conditions according to the instructions in the handbook.

10. Transfer up to 700 µl of the mix to a MB RNA Spin Column (pink) placed in a 2 ml collection tube. Centrifuge at 15,000 x *g* for 1 min. Discard the flow-through.

    Note: If the volume of the mixture exceeds 700 µl, centrifuge successive aliquots in the same MB RNA Spin Column. Discard the flow-through after each centrifugation.

DNA and RNA washing and elution

11. Add 650 µl Solution EA to a MB DNA Spin Column (white) and a MB RNA Spin Column (pink) and centrifuge at 15,000 x *g* for 1 min. Discard the flow-through.
12. Add 500 µl Solution C5. Centrifuge at 15,000 x *g* for 1 min.
13. Discard flow-through and place the MB RNA and DNA Spin Columns into clean 2 ml collection tubes (provided). Centrifuge at 20,000 x *g* (or full speed) for 1 min.
14. Place the MB RNA and DNA Spin Columns into clean 1.5 ml Elution Tubes (provided).
15. Add 100 µl RNase-free water to the center of the white filter membrane.
16. Incubate at room temperature for at least 1 min.
17. Centrifuge at 15,000 x *g* for 1 min. Discard the MB RNA and DNA Spin Columns. The RNA and DNA are now ready for any downstream applications.

Document Revision History

Date	Changes
02/2022	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.