April 2022

Quick-Start Protocol

RNeasy® PowerFecal® Pro Kit

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

The RNeasy PowerFecal Pro Kit is for the isolation of microbial RNA from stool samples.

Further information

- *RNeasy PowerFecal Pro Kit Handbook*: **www.qiagen.com/HB-2918**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Ensure that the PowerBead Pro Tubes rotate freely in the centrifuge without rubbing.
- Prepare DNase I stock enzyme by adding 550 µl RNase-free Water to the DNase I (RNase-free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 50 µl portions and store at –30 to –15°C for long-term storage. Avoid freeze/thaw more than three times. To prepare DNase I Solution, thaw and combine 5 µl DNase I stock enzyme with 45 µl DNase Digestion Solution per prep. DNase I is sensitive to physical denaturation; do not vortex resuspended DNase I.
- Perform all centrifugation steps at room temperature.
- Refer to the *RNeasy PowerFecal Pro Kit Handbook* for optimal homogenization method in step 3.

Procedure

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) or QIAzol® Lysis Reagent (cat. no. 79306) to the PowerBead Pro Tube and vortex briefly to mix.
3. Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 24 (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 the *RNeasy PowerFecal Pro Kit Handbook*.
4. Centrifuge the PowerBead Pro Tube at 15,000 x *g* for 1 min.
5. Transfer the supernatant to a clean 2 ml microcentrifuge tube (provided).

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

   Note: If desired, up to 650 µl supernatant can be used and mixed with Solution EA in a ratio of 1:1. 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.
8. Add 300 µl of Solution EA. Vortex briefly to mix.
9. Load 600 µl supernatant-EA mix into an MB RNA Spin Column and centrifuge at 15,000 x *g* for 1 min. Discard the flow-through.
10. Add 650 µl Solution EA and centrifuge at 15,000 x *g* for 1 min.

11. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided). Add 50 µl DNase I Solution to the center of the Spin Column (prepared by mixing 45 µl DNase Digestion Solution and 5 µl DNase I stock enzyme; see "Notes before starting").
12. Incubate at room temperature for 15 min. Add 650 µl Solution EA and centrifuge at 15,000 x *g* for 1 min.
13. Discard flow-through. Add 500 µl Solution C5. Centrifuge at 15,000 x *g* for 1 min.
14. Discard flow-through and place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided). Centrifuge at 20,000 x *g* (or full speed) for 1 min.
15. Place the MB RNA Spin Column into a clean 1,5 ml Elution Tube (provided).
16. Add 100 µl RNase free water to the center of the white filter membrane.
17. Incubate at room temperature for at least 1 min.
18. Centrifuge at 15,000 x *g* for 1 min. Discard the MB RNA Spin Column. The RNA is now ready for any downstream application.

Document Revision History

Date	Changes
04/2022	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.