DNA Extraction from Fecal Samples

DNeasy® PowerSoil® Pro 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). Further information DNeasy® PowerSoil® Pro Kit Handbook: www.qiagen.com/HB-2495 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.
- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- Perform all centrifugation steps at room temperature (15–25°C).

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. Add up to 250 mg of soil and 800 µl of Solution CD1. Vortex briefly to mix.
2. Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5–2 ml tubes (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.

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

Note: For more information about other bead beating methods, see the “Protocol: Detailed” section of DNeasy® PowerSoil® Pro Kit Handbook.

Use a TissueLyser II. Place the PowerBead Pro Tube into the TissueLyser Adapter Set 2 x 24 (cat. no. 69982) or 2 ml Tube Holder (cat. no. 11993) and Plate Adapter Set (cat. no. 11990). Fasten the adapter into the instrument and shake for 5 min at speed 25 Hz. Reorient the adapter so that the side that was closest to the machine body is now furthest from it. Shake again for 5 min at a speed of 25 Hz.

Note: Vortexing/shaking is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from step 1 and mechanical shaking introduced at this step. Randomly shaking the beads in the presence of disruption agents will cause the beads to collide with microbial cells and lead to the cells breaking open.

3. Centrifuge the PowerBead Pro Tube at 15,000 x g for 1 min.
4. Transfer the supernatant to a clean 2 ml Microcentrifuge Tube (provided). Note: Expect 500–600 µl. The supernatant may still contain some soil particles.
5. Add 200 µl of Solution CD2 and vortex for 5 s.
6. Centrifuge at 15,000 x g for 1 min at room temperature. Avoiding the pellet, transfer up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube (provided). Note: Expect 500–600 µl.
7. Add 600 µl of Solution CD3 and vortex for 5 s.
8. Load 650 µl of the lysate onto an MB Spin Column and centrifuge at 15,000 x g for 1 min.
9. Discard the flow-through and repeat step 8 to ensure that all of the lysate has passed through the MB Spin Column.

10. Carefully place the MB Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the MB Spin Column.
11. Add 500 µl of Solution EA to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.
12. Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.
13. Add 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.
14. Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube (provided).
15. Centrifuge at up to 16,000 x g for 2 min. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided).
16. Add 50–100 µl **(our application uses 60ul)** of Solution C6 to the center of the white filter membrane.
17. Centrifuge at 15,000 x g for 1 min.
18. Repeat Step 16 by pipetting the flow through volume directly back onto the spin column.
19. Centrifuge at 15,000 x g for 1 min. Discard the MB Spin Column.

The DNA is now ready for downstream applications. Note: We recommend storing the DNA frozen (–30 to –15°C or –90 to –65°C) as Solution C6 does not contain EDTA. To concentrate DNA, please refer to the Troubleshooting Guide.