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DNeasy® PowerSoil® Pro Kit

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We are still developing and optimizing this protocol

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Abstract

QIAGEN DNeasy ® PowerSoil ® Pro Kit Protocol adapted for microbiome research with students and during courses.

Attachments

PDF

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465KB

Guidelines

The DNeasy PowerSoil Pro Kit does not require homogenization using a high-velocity bead beater. However, if the microorganism of interest requires stronger homogenization than provided by a vortex, or if using a bead beater is desired, the DNeasy PowerSoil Pro Kit contains bead tubes suitable for high-powered bead beating and may be used in conjunction with the PowerLyzer® 24 Homogenizer (110/220V) (cat. no. 13155) or the TissueLyser II (cat. no. 85300) using a 2 ml Tube Holder Set (cat no. 11993).

Before start

- 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).

Suspend Sample

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

Note: After the sample has been loaded into the PowerBead Pro Tube, the next step is a homogenization and lysis procedure. The PowerBead Pro Tube contains a buffer that will (a) help disperse the soil particles, (b) begin to dissolve humic acids, and (c) protect nucleic acids from degradation. Gentle vortexing mixes the components in the PowerBead Pro Tube and begins to disperse the sample in the buffer.

1.1 For Fungal Samples. Place the culture in a 2 ml Collection Tube and centrifuge at 10,000 x g for 30 seconds at room temperature.

Decant the supernatant and centrifuge the pellet again at 10,000 x g for 30 seconds at room temperature until the media supernatant is completely removed.

Resuspend the cell pellet in 800 µl of Solution CD1 and transfer to a PowerBead Pro Tube.

2 Add up to 250 mg of soil and 800 µl of Solution CD1.

2.1 Vortex briefly to mix.

3 Mix samples thoroughly. Secure the PowerBead Pro Tube horizontally with a Vortex Adapter on a Vortex Genie 2 (suggested) for 1.5–2 ml tubes.

Vortex at maximum speed for 10 min.

Note: If using Vortex Adapter for more than 12 preps simultaneously, increase the vortexing time to 15-20 min.
Note: Be careful using tape, which can become loose and affect samples.

4 Centrifuge the PowerBead Pro Tube at 15,000 x g for 1 min.

5 Transfer the supernatant (the liquid top layer in the tube) to a clean 2 ml Microcentrifuge Tube (in Kit).

Note: Expect a volume around 500–600 µl. The supernatant may still contain some soil particles.

Isolate DNA

6 Add 200 µl of Solution CD2.

Note: Solution CD2 contains IRT (Inhibitor Removal Technology ®), which is a reagent that can precipitate non-DNA organic and inorganic material including humic substances, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

6.1 Vortex for 5 s.

7 Centrifuge at 15,000 x g for 1 min.

8 Pipette up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube (in Kit).
Avoid pipetting the pellet.

9 Add 600 µl of Solution CD3.

Note: Solution CD3 is a high-concentration salt solution. Because DNA binds tightly to silica at high salt concentrations, Solution CD3 will adjust the DNA solution salt concentration to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the MB Spin Column filter membrane.

9.1 Vortex for 5 s.

10 Pipette 650 µl of the lysate (the current contents of the tub) onto an MB Spin Column.

10.1 Centrifuge at 15,000 x g for 1 min.

Note: DNA is selectively bound to the silica membrane in the MB Spin Column in the presence of high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.

10.2 Discard the flow-through.

11 Pipette the rest of the lysate to ensure that all of the lysate has passed through the MB Spin Column.

11.1 Centrifuge at 15,000 x g for 1 min.

11.2 Discard the flow-through.

Wash Sample and remove contaminants

12 Add 500 µl of Solution EA to the MB Spin Column.

Note: Solution EA is a wash buffer that removes protein and other non-aqueous contaminants from the MB Spin Column filter membrane.

12.1 Centrifuge at 15,000 x g for 1 min.

12.2 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.

Note: Solution C5 is an ethanol-based wash solution used to further clean the DNA that is bound to the silica filter membrane in the MB Spin Column. This wash solution removes residual salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.

13.1 Centrifuge at 15,000 x g for 1 min.

13.2 Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube (in Kit).

14 Centrifuge at up to 16,000 x g for 2 min.

Note: This spin removes residual Solution C5. It is critical to remove all traces of Solution C5 because the ethanol in it can interfere with downstream DNA applications, such as PCR, restriction digests, and gel electrophoresis.

15 Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (in Kit).

Elute DNA

16 Add 100 µl of Solution C6 (or Solution EB) to the center of the white filter membrane.

Note: Pipetting the solution in the center of the small white membrane will make sure the entire membrane is wet. This will result in a more efficient and complete release of the DNA from the MB Spin Column filter membrane. As Solution C6 passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution C6 (10 mM Tris), which lacks salt.

17 Centrifuge at 15,000 x g for 1 min. Discard the MB Spin Column.

18 Store the DNA in either a –20°C or –80°C for long term storage.