Quick-Start Protocol August 2016

DNeasy® PowerLyzer® PowerSoil® Kit

The DNeasy PowerLyzer PowerSoil Kit can be stored at room temperature (15–25°C) until the expiry date printed on the box label.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Perform all centrifugation steps at room temperature (15–25°C)
- If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.
- Shake to mix Solution C4 before use

1. Add up to 0.25 g of soil sample to the PowerBead Tube provided.
2. Add 750 µl of PowerBead Solution to the PowerBead Tube.
3. Add 60 µl of Solution C1 and invert several times or vortex briefly.
4. Bead beating options:
   A. **PowerLyzer 24 homogenizer:** Place the PowerLyzer Glass Bead Tubes into the tube holder for the PowerLyzer 24. The PowerBead Tubes must be balanced on the tube holder. Run the samples for a time and RPM suitable for your soil type.
   Note: For clay soils, 4,000 RPM for 45 s is the best starting point. For loose, granular and high organic soils, 2,500 RPM for 45 s will provide an optimal result
   B. **Vortex:** Secure the PowerBead Tubes horizontally using a Vortex Adapter tube holder (cat. no. 13000–V1–24). Vortex at maximum speed for 10 min.
   Note: If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5–10 min.
5. Centrifuge Bead Tubes at 10,000 x *g* for 30 s. **Do not** exceed 10,000 x *g*.
   Note: Centrifuge for 3 min at 10,000 x *g* for clay soils or if your soil is not completely pelleted after 30 s.
6. Transfer the supernatant to a clean 2 ml collection tube (provided).

Note: Expect 400–500 µl. Supernatant may still contain some soil particles.

7. Add 250 µl of Solution C2 and vortex for 5 s. Incubate at 2–8°C for 5 min.

   Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step.

8. Centrifuge the tubes for 1 min at 10,000 x *g*. Avoiding the pellet, transfer up to 600 µl of supernatant to a clean 2 ml collection tube (provided).

9. Add 200 µl of Solution C3 and vortex briefly. Incubate at 2–8°C for 5 min.

   Note: You can skip the 5 min incubation. However, if you have already validated the PowerSoil extractions with the incubation we recommend you retain the step.

10. Centrifuge the tubes for 1 min at 10,000 x *g*. Avoiding the pellet, transfer up to 750 µl of supernatant into a clean 2 ml collection tube (provided).

11. Add 1200 µl of Solution C4 to the supernatant and vortex for 5 s.

12. Load 675 µl of the supernatant onto a MB Spin Column and centrifuge at 10,000 x *g* for 1 min. Discard the flow through and add an additional 675 µl of supernatant.

13. Centrifuge at 10,000 x *g* for 1 minute. Load the remaining supernatant onto the MB Spin Column and centrifuge at 10,000 x *g* for 1 min.
    Note: A total of three loads for each sample processed are required.

14. Add 500 µl of Solution C5 and centrifuge for 30 s at 10,000 x *g*.

15. Discard the flow through. Centrifuge again for 1 min at 10,000 x *g*.

16. Carefully place spin filter in a clean 2 ml collection tube (provided). Avoid splashing any Solution C5 onto the MB Spin Column.

17. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, you may use sterile DNA-Free PCR Grade Water or TE buffer (cat. no. 17000-10).

18. Centrifuge for 30 s at 10,000 x *g*. Discard the MB Spin Column

19. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20° to –80°C) as Solution C6 does not contain EDTA. To concentrate DNA see the Hints & Troubleshooting Guide.