Quick-Start Protocol November 2016

DNeasy® PowerLyzer® Microbial Kit

The DNeasy PowerLyzer Microbial 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

- The PowerLyzer 24 may cause marring of labels on the tops of the Glass MicroBead Tubes. To ensure proper sample identification, label sides and tops of the tubes.
- If Solution SL has precipitated, heat at 60°C until the precipitate has dissolved.
- Shake to mix Solution SB before use.

1. Add 1.8 ml of microbial (bacteria, yeast) culture to a 2 ml collection tube (provided) and centrifuge at 10,000 x *g* for 30 s at room temperature. Decant the supernatant and spin the tubes at 10,000 x *g* for 30 s at room temperature and completely remove the media supernatant with a pipette tip.
   Note: Based on the type of microbial culture, it may be necessary to centrifuge longer than 30 seconds.
2. Resuspend the cell pellet in 300 µl of PowerBead Solution and gently vortex to mix. Transfer resuspended cells to a PowerBead Tube Glass, 0.1 mm.
3. Add 50 µl of Solution SL to the Glass PowerBead Tube.
   Note: To increase yields, to minimize DNA shearing or for cells that are difficult to lyse, refer to the Troubleshooting Guide.
4. Homogenization options:

   a) PowerLyzer 24 Homogenizer: Balance PowerBead Tubes in the tube holder for the PowerLyzer 24. Homogenize for 5 min at 2000 RPM.
   Note: Depending on the sample, you can homogenize at a higher speed for less time.

b) Vortex: Secure PowerBead Tube horizontally using the Vortex Adapter tube holder (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
Note: To minimize DNA shearing, refer to Troubleshooting Guide.

5. Make sure the PowerBead Tubes rotate freely in the centrifuge without rubbing. Centrifuge the tubes at a **maximum** of 10,000 x *g* for 30 s at room temperature.
6. Transfer the supernatant to a clean 2 ml collection tube (provided).
   Note: Expect 300 to 350 µl of supernatant.
7. Add 100 µl of Solution IRS and vortex for 5 s. Incubate at 4°C for 5 min.
8. Centrifuge 10,000 x *g* for 1 min at room temperature.
9. Avoiding the pellet, transfer all of the supernatant to a 2 ml collection tube (provided).
   Note: Expect approximately 450 µl of supernatant. A small carryover of glass beads is possible. This will not affect the results.
10. Add 900 µl of Solution SB to the supernatant and vortex for 5 s.
11. Load about 700 µl into a MB Spin Column and centrifuge at 10,000 x *g* for 30 s at room temperature. Discard the flow-through, add the remaining supernatant to the MB Spin Column, and centrifuge at 10,000 x *g* for 30 s at room temperature.
    Note: Each sample processed will require 2–3 loads. Discard all flow-through.
12. Add 300 µl of Solution CB and centrifuge at 10,000 x *g* for 30 s at room temperature.
13. Discard the flow-through and centrifuge at 10,000 x *g* for 1min at room temperature.
14. Being careful not to splash liquid on the spin filter basket, place MB Spin Column in a new 2 ml collection tube (provided).
15. Add 50 µl of Solution EB to the center of the white filter membrane.
16. Centrifuge at 10,000 x *g* for 30 s at room temperature.
17. Discard the MB Spin Column. The DNA is now ready for downstream applications.
    Note: We recommend storing DNA frozen (–20° to –80°C) as Solution EB does not contain EDTA.