Quick-Start Protocol February 2017

DNeasy® UltraClean® Microbial Kit

The DNeasy UltraClean 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

- If Solution SL has precipitated, heat at 55°C for 5–10 min.
- 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 again at 10,000 x *g* for 30 s at room temperature. Completely remove the supernatant with a pipette tip.
   Note: Depending on the type of microbial culture, it may be necessary to centrifuge longer than 30 s.
2. Resuspend the cell pellet in 300 µl of PowerBead Solution and gently vortex to mix. Transfer resuspended cells to PowerBead Tube.
3. Add 50 µl of Solution SL to the PowerBead Tube.
   Note: To increase yields, to minimize DNA shearing, or for difficult cells, refer to the Troubleshooting Guide.
4. Secure PowerBead Tubes horizontally using the Vortex Adapter tube holder (cat. no. 13000-V1). Vortex at maximum speed for 10 min.
5. Make sure the 2 ml 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 300–350 µl of supernatant.
7. Add 100 µl of Solution IRS to the supernatant and vortex for 5 s. Incubate at 4°C for 5 min.
8. Centrifuge the tubes at 10,000 x *g* for 1 min at room temperature.
9. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml collection tube (provided).
   Note: Expect 450 µl of supernatant.
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 again at 10,000 x *g* for 30 s at room temperature.
    Note: Each sample processed will require 2–3 loads. Discard all flow-through liquid.
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. Centrifuge at 10,000 x *g* for 1 min at room temperature.
14. Place the MB Spin Column in a new 2 ml collection tube (provided).
    Note: Be careful not to splash any of the liquid on the Spin Filter basket.
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.