Quick-Start Protocol November 2016

# DNeasy® UltraClean® 96 Microbial Kit

The DNeasy UltraClean 96 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

- This protocol assumes you will be processing 192 samples (2 x 96-well preps). If you plan to process fewer than 192 samples, divide the samples between two plates such that they are balanced. See the Troubleshooting Guide for more information.
- If Solution SL has precipitated, heat at 60°C until precipitate dissolves.
- Shake to mix Solution SB before use.

1. Dispense liquid culture into a clean 2 ml collection plate and cover with Sealing Tape.
2. Centrifuge at 4500 x *g* for 12 min. Discard tape and remove all supernatant.
3. Add 300 µl of PowerBead Solution and apply new Sealing Tape. Resuspend the cell pellet by vortexing.
4. Centrifuge the PowerBead DNA Plate at 4500 x *g* for 3 min to bring down all the beads.
5. Remove and discard Elution Sealing Mat from the PowerBead DNA Plate and transfer the resuspended cells from step 3 to the PowerBead DNA Plate.
6. Add 60 µl of Solution SL and seal PowerBead Plate securely with new Sealing Mat.
7. Place the securely-sealed PowerBead Plate in a TissueLyser II (cat. no. 85300).
   **Note:** Final order of components: Adapter plate (cat. no. 11990), Sealing Mat, PowerBead Plate, adapter plate.
8. Shake at speed 20 Hz for 5 min. Re-orient plates so that the side that was closest to the machine body is now furthest from it and shake again at speed 20 Hz for 5 min.

9. Centrifuge at 4500 x *g* for 6 min at room temperature.
10. Remove and discard Sealing Mat. Transfer supernatant to a clean 1 ml collection plate. **Note:** Supernatant may still contain some beads.
11. Add 100 µl of Solution IRS. Apply Sealing Tape and vortex for 5 s.
12. Incubate at 2°–8°C for 10 min. Centrifuge the plate at 4500 x *g* for 9 min.
13. Avoiding the pellet, transfer supernatant to a clean 2 ml collection plate.
14. Add 800 µl of Solution SB to wells containing supernatant. Pipet up and down to mix.
15. Place a QIAamp 96 Plate onto an S-Block.
16. Transfer approximately 650 µl of supernatant to the QIAamp 96 Plate.
17. Apply Airpore tape sheet and centrifuge at 4500 x *g* for 3 min.
18. Discard flow-through and replace the S-Block beneath the QIAamp 96 Plate. Discard the Airpore Tape Sheet.
19. Repeat steps 16–18 until all the supernatant has been processed.
20. Add 400 µl of Solution CB to the QIAamp 96 Plate and apply Airpore Tape Sheet.
21. Centrifuge at 4500 x *g* for 3 min.
22. Discard Airpore Tape Sheet and flow-through. Place the QIAamp 96 Plate on the same S-Block and add another 400 µl of Solution CB. Apply a new piece of Airpore Tape Sheet and centrifuge at 4500 x *g* for 3 min.
23. Discard flow-through and place the QIAamp 96 Plate on the same S-Block.
24. Centrifuge at 4500 x *g* for 6 min. Carefully place the QIAamp 96 Plate on an Elution Microtube Rack being careful not to splash any Solution CB onto the QIAamp 96 Plate.
25. Discard the flow-through. The S-Block can be re-used.
26. Remove and discard Airpore Tape Sheet. Air dry for 10 min at room temperature.
27. Add 100 µl of Solution EB to the centers of the QIAamp 96 Plate filter membranes.
28. Apply Airpore Tape Sheet to the QIAamp 96 Plate. Centrifuge at 4500 x *g* for 3 min.
29. Cover wells of the Elution Microtube Rack with Strip Caps (provided). The DNA in the Elution Microtube Rack is now ready for downstream applications.