Quick-Start Protocol June 2016

DNeasy® PowerSoil® HTP 96 Kit, centrifuge protocol

The DNeasy PowerSoil HTP 96 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 C1 has precipitated, heat at 60°C until precipitate dissolves.
- To prepare Solution C5-D, add equal amount (120 ml for 4 prep kit or 360 ml for 12 prep kit) of 100% ethanol to Solution C5-D. Mix well.
- Please wear gloves at all times.

1. Remove the Square Well Mat from the Bead Plate. Add up to 0.25 g of soil sample.
   Note: Avoid cross contamination between sample wells. This is an appropriate stopping point and you can store the Bead Plate at 2–8°C covered with the Square Well Mat.
2. Add 750 µl of PowerBead Solution to the wells of the Bead Plate.
3. Add 60 µl of Solution C1. Secure the Square Well Mat tightly to the plate.
4. Place Bead Plate with mat securely fastened between 2 adapter plates (Cat. # 11990) on a 96-Well Plate Shaker or TissueLyser II (Cat. # 85300).
5. Shake at speed 20 Hz for 10 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 10 min.
6. Centrifuge at room temperature for 6 min at 4500 x *g*.
7. Discard the Square Well Mat. Transfer the supernatant to a clean 1 ml collection plate.
   Note: The supernatant may still contain some soil particles.
8. Add 250 µl of Solution C2.
9. Apply sealing tape to plate. Vortex for 5 s. Incubate at 2–8°C for 10 min.
   Note: You can skip the 10 min incubation. However, if you have already validated the DNeasy PowerSoil extractions with the incubation we recommend you retain the step.

10. Centrifuge the plate at room temperature for 6 min at 4500 x *g*. Discard sealing tape.
11. Avoiding the pellet, transfer entire volume of supernatant to a new 1 ml collection plate.
12. Apply sealing tape to plate and repeat steps 10–11 once. Then move on to step 13.
13. Add 200 µl of Solution C3 and repeat steps 9–11 once. Then apply sealing tape to the plate and centrifuge at room temperature for 6 min at 4500 x *g*.
14. Transfer no more than 650 µl of supernatant to a 2 ml collection plate.
15. Add 650 µl of Solution C4 to each well of the plate. Repeat (to add 1300 µl total).
    Note: You can pause here and store the samples covered with sealing tape at 2–8°C.
16. Pipet samples up and down to mix. Place a spin plate onto an S-block.
17. Load approximately 650 µl into each well of the spin plate and seal plate with an AirPore Tape Sheet.
18. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through and place the spin plate back on the same S-block. Discard the AirPore Tape Sheet.
19. Repeat steps 17 and18 until all the supernatant has been processed. Discard final flow-through.
20. Place the spin plate back on the same S-block.
21. Add 500 µl of Solution C5-D to each well of the spin plate. Seal each plate with an AirPore Tape Sheet.
22. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the flow-through and place the spin plate back on the same S-block. Seal plate with an AirPore Tape Sheet.
23. Centrifuge again at room temperature for 5 min at 4500 x *g*. Discard flow-through.
24. Carefully place the spin plate onto a racked Elution Microtubes CL. Discard the AirPore Tape Sheet.
25. Allow to air dry for 10 min at room temperature.
26. Add 100 µl of Solution C6 to the center of each well. Seal plate with an AirPore Tape Sheet.
27. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the AirPore Tape Sheet.
28. Seal Elution Microtubes with the caps provided.