DNeasy® 96 PowerSoil® Pro Kit

Solution CD2 should be stored at 2–8°C upon arrival. All other reagents and kit components should be stored at room temperature (15–25°C) until the expiry date printed on the kit label.

Further information

- *DNeasy 96 PowerSoil Pro Kit Handbook*: **www.qiagen.com/HB-2675**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Refer to the *DNeasy PowerBead Pro Plate Quick-Start Protocol* for optimal homogenization using the TissueLyser II.
- If Solution CD3 has precipitate, heat at 60°C until precipitate dissolves.
- Use a 96-well plate rotor and perform all centrifugation steps at room temperature.
- Use extra-long pipette tips (1000–1250 µl) for collection microtubes, racked (CMTRs).

1. Centrifuge the PowerBead Pro Plate briefly to ensure that the beads have settled at the bottom of the well.
2. Remove and discard the square well mat from the PowerBead Pro Plate. Add up to 0.25 g of soil or 0.1 g of stool sample and 800 µl of Solution CD1.
3. Make sure to remove any residual liquid on top of the plate and seal the plate with sealing film (provided).
   Note: A strong seal is essential to prevent leakage during disruption in the TissueLyser II. Usage of a mechanical plate sealer can be advantageous for a consistent and a uniform seal.
   Homogenize samples thoroughly using the TissueLyser II:
   Place a silicone compression mat on top of the sealing film, then place the sealed plate and mat between 2 Adapter Plate Sets (cat.no.11990). Shake for 5 min at 25 Hz.
   Re-orient the plates so that the side that was closest to the machine body is now furthest from it. Shake again for 5 min at 25 Hz.
   Important: When using this assembly, do not exceed the recommended disruption time and setting of 2 x 5 min at 25 Hz, because extended processing might lead to leakage.
4. Centrifuge the PowerBead Plate at room temperature for 6 min at 4500 x *g*.
5. Discard the sealing film. Transfer the supernatant to the collection microtubes.
   Note: Expect a volume of 500–600 µl. The supernatant may still contain soil particles.

6. Add 200 µl of Solution CD2. Seal the collection microtubes with the caps provided and vortex.
   Note: If processing samples with very high inhibitor content, use 250 µl of Solution CD2.
7. Centrifuge the plate at room temperature for 6 min at 4500 x *g*.
8. Transfer up to 700 µl of supernatant to an S-Block.
   Note: Expect a volume of 500–600 µl.
9. Add 600 µl of Solution CD3 to each well of the plate. Pipet samples up and down to mix. Place a QIAamp 96 Spin Plate onto a new S-Block.
10. Load approximately 650 µl into each well of the spin plate and seal the plate with a sealing tape.
11. 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 sealing tape.
12. Repeat steps 10 and 11 until all the supernatant has been processed. Discard the final flow-through.
13. Place the spin plate back on the same S-Block.
14. Add 500 µl of Solution EA to each well of the spin plate and seal the plate with a sealing tape.
15. 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.
16. Add 500 µl of Solution C5 to the spin plate and seal the plate with a sealing tape. Centrifuge for 3 min at 4500 x *g*.
17. Discard the flow-through (remove any trace of flow-through from the S-Block) and place the spin plate (still sealed from step 16) into the same S-Block.
18. Centrifuge again at room temperature for 5 min at 4500 x *g*. Discard the flow-through.
19. Carefully place the spin plate onto new collection microtubes. Discard the sealing tape.
20. Allow to air dry for 10 min at room temperature.
21. Add 100 µl of Solution C6 to the center of each well. Seal the plate with a sealing tape.
22. Centrifuge at room temperature for 3 min at 4500 x *g*. Discard the sealing tape.
23. Seal the collection microtubes with the caps provided. The DNA is now ready for downstream applications.

Document Revision History

Date	Changes
07/2021	Added additional information about storage and statements about using a mechanical plate sealer and not exceeding the recommended disruption time and setting on procedure step 3.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.