Quick-Start Protocol February 2017

DNeasy® PowerPlant® Pro HTP 96 Kit

RNase A should be stored at 2–8°C. All other components of the DNeasy PowerPlant Pro 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 SL contains precipitates, heat at 37–55°C to dissolve precipitates.
- Add 106 ml of 100% ethanol **(user provided)** to Solution IW before starting to prepare the final wash solution. Check the box and write the date on the label.
- For each prep, add 3 µl of RNase A to PowerBead Solution before starting. For each 96 well plate, add 300 µl of RNase A to 45 ml of PowerBead Solution.

1. Add up to 50 mg of wet plant material (or up to 10 mg of dry material) to your chosen bead homogenization plate. Add 450 µl of RNase A/PowerBead Solution (if your sample is low in phenolics) or 410 µl of RNase A/PowerBead Solution and 40 µl of Phenolic Separation Solution (if your sample is high in phenolics).
   Note: We recommend that you cut the tissue into small pieces before loading into the bead homogenization plate. For tough plants or seeds pre-grind the material with a mortar and pestle.
2. Add 50 µl of Solution SL to each sample. Place bead homogenization plate with mat securely fastened between 2 Adapter Plates (cat. no. 11990) and place on a TissueLyser II (cat. no. 85300). Refer to the adapter plate protocol for proper placement.
3. Shake at speed 20 for 8 min. Remove the plate and re-position so that the side closest to the machine body is now furthest from it, and shake again at speed 20 for 8 min.
   Note: For samples other than soft leaf tissue, optimization of bead beating settings specific to your sample type is required.
4. Centrifuge at 4500 x *g* for 9 min. Remove and discard sealing mat. Transfer supernatant to a clean 1 ml Collection Plate (supernatant may still contain particles and debris).
5. Add 175 µl of Solution IR and apply Sealing Tape to plate. Vortex for 5 s.
   Note: For problematic samples, you can add up to 250 µl of Solution IR.

6. Incubate the 1 ml Collection Plate at 4°C for 10 min. Centrifuge at 4500 x *g* for 9 min. Remove and discard the Sealing Tape.
7. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Plate.
8. Add 600 µl of Solution PB to the first row of the 2 ml Collection Plate containing the supernatant and pipet up and down to mix. Repeat for all remaining rows.
9. Add 600 µl of 100% ethanol (**user provided**) and pipette up and down to mix.
10. Seal any unused wells of a QIAamp 96 Plate with AirPore Tape Sheet and place the QIAamp 96 Plate onto an S-Block.
11. Carefully transfer half of each sample (from the 2ml Collection Plate) to the QIAamp 96 Plate. Apply AirPoreTape Sheet and centrifuge at 4500 x *g* for 5 min.
12. Repeat Step 11 until all the supernatant has been processed (two loads).
13. Discard the flow through from the S-Block and replace the same S-Block beneath the QIAamp 96 Plate. Discard the AirPoreTape Sheet.
14. Make sure that 100% ethanol has been added to the bottle containing Solution IW. Add 500 µl of Solution IW to each well of the QIAamp 96 Plate.
15. Apply AirPore Tape Sheet. Centrifuge at 4500 x *g* for 3 min.
16. Add 500 µl of 100% ethanol (**user provided**) to each well of the Spin Plate. Apply a new piece of AirPore Tape Sheet and centrifuge for at 4500 x *g* for 3 min.
17. Discard the flow through and replace same S-Block beneath the QIAamp 96 Plate. Centrifuge at 4500 x *g* for 7 min.
18. Carefully place the QIAamp 96 Plate on an Elution Microtube Rack. Remove the AirPore Tape Sheet and discard. Air dry for 10 min at room temperature.
19. Add 100 µl of Solution EB to the center of the white filter membrane in the QIAamp 96 Plate wells. Apply a new piece of AirPoreTape Sheet. Incubate for 2 min at room temp.
20. Centrifuge at 4500 x *g* for 3 min. Remove and discard the AirPoreTape Sheet. If all 96 wells were utilized you can discard the entire QIAamp 96 Plate.
21. Seal the Elution Microtubes with the Elution Microtube Caps (provided). The DNA is now ready for downstream applications.