DNeasy® 96 Plant Kit

The DNeasy 96 Plant Kit (cat. no. 69181) can be stored at room temperature (15–25°C) for up to 1 year.

For more information, please refer to the *DNeasy Plant Handbook* and the *TissueLyser Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- This protocol is for purifying DNA from 2 x 96 samples of fresh plant tissue.
- Ensure that you are familiar with operating the TissueLyser and the QIAGEN® 96-Well-Plate Centrifugation System.
- Perform all centrifugation steps at room temperature (15–25°C).
- If necessary, redissolve any precipitates in Buffer AP1 and Buffer AW1 concentrates.
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates.
- Preheat Buffer AP1 to 65°C.
- Prepare a fresh working lysis solution: For 2 x 96 samples, combine 90 ml Buffer AP1, 225 µl RNase A, and 225 µl Reagent DX.

1. Place up to 50 mg leaves into each tube in 2 collection microtube racks.
2. Add 1 tungsten carbide bead to each collection microtube.
3. Pipet 400 µl working lysis solution into each collection microtube. Tightly seal the microtubes using the caps provided.
4. Assemble each rack of collection microtubes into the TissueLyser.
5. Grind the sample for 1.5 minutes at 30 Hz.

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6. Reassemble the racks so that the collection microtubes nearest the TissueLyser in steps 4 and 5 are now furthest from the TissueLyser.
7. Grind the samples for another 1.5 min at 30 Hz.
8. Centrifuge to collect any solution from the caps.
9. Add 130 µl Buffer P3 to each collection microtube and reseal using new caps.
10. Place a clear cover over each rack and shake vigorously up and down for 15 s. Centrifuge to collect any solution from the caps.
11. Incubate the collection-microtube racks for 10 min at –20°C.
12. Centrifuge the collection-microtube racks for 5 min at 3800 x *g* (6000 rpm).
13. Transfer 400 µl of each supernatant to a new collection microtube.
14. Add 600 µl of Buffer AW1 to each sample. Close microtubes with new caps.
15. Place a clear cover over each rack and shake vigorously up and down for 15 s. Centrifuge to collect any solution from the caps.
16. Place 2 DNeasy 96 plates on top of S-Blocks. Mark the DNeasy 96 plates for later sample identification.
17. Transfer 1 ml of each sample to each well of the DNeasy 96 plates.
18. Seal each DNeasy 96 plate with an AirPore Tape Sheet. Centrifuge for 4 min at 3800 x *g*. If lysate remains in the DNeasy 96 plates after centrifugation, centrifuge for another 4 min.
19. Remove the tape. Add 800 µl Buffer AW2 to each sample.
20. Centrifuge for 15 min at 3800 x *g* without tape to dry the membranes.
21. Place each DNeasy 96 plate on a new Elution Microtubes RS rack.
22. Add 100 µl Buffer AE and seal with new AirPore Tape Sheets. Incubate for 1 min at room temperature (15–25°C). Centrifuge for 2 min at 3800 x *g*.
23. Repeat step 22. Seal the Elution Microtubes RS with new caps to store DNA.