DNeasy® Plant 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 box label.

Further information

- DNeasy Plant Pro Handbook: www.qiagen.com/HB-2552
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.aigaen.com

Notes before starting

- If Buffer APP contains precipitates, heat at 60°C until precipitate dissolves.
- Perform all centrifugation steps at room temperature (15–25°C).
- Refer to kit handbook for optimal homogenization method in step 2.
 - Add 5-100 mg of fresh or frozen plant tissue and 500 µl of Solution CD1 to a 2 ml tissue disruption tube. Vortex briefly to mix.
 - **Note**: If your sample is high in phenolic compounds, add 450 μ l Solution CD1 and 50 μ l Solution PS.
 - 2. Homogenize using one of these methods:
 - 2a. **Vortex**: Secure tissue disruption tubes to a Vortex Adapter (cat. no. 13000-V1-24) and vortex at maximum speed for 10 min.
 - 2b. **TissueLyser II**: Most plant samples can be lysed with the TissueLyser II, using the TissueLyser Adapter Set 2 x 24: Place samples in the TissueLyser II and run at 24 Hz for 2 min. Reorient the adapter so the side closest to the machine body becomes furthest from it, and then run the TissueLyser again at 24 Hz for another 2 min.
 - 2c. PowerLyzer® 24 Homogenizer: Tissue disruption tubes must be properly balanced in the tube holder of the PowerLyzer 24 Homogenizer. Homogenize the tissue for 1 cycle at the appropriate speed depending on sample type for 2 min.
 - 3. Centrifuge the tissue disruption tubes at $12,000 \times g$ for 2 min.



- 4. Transfer the supernatant to a clean 1.5 ml microcentrifuge tube (provided).

 Note: Expect 350–450 µl. The supernatant may still contain some plant particles.
- 5. Add 200 μ l Solution CD2 and vortex for 5 s.

Note: For problematic samples, add 250 µl Solution CD2.

6. Centrifuge at $12,000 \times g$ for 1 min at room temperature. Avoiding the pellet, transfer the supernatant to a clean 1.5 ml microcentrifuge tube (provided).

Note: Expect 400–500 μl.

- 7. Add 500 µl of Buffer APP and vortex for 5 s.
- 8. Load 600 μ l lysate onto an MB Spin Column. Centrifuge at 12,000 x g for 1 min.
- 9. Discard the flow-through and repeat step 8 to ensure that all of the lysate has passed through the MB spin column.
- 10. Place the MB spin column into a clean 2 ml collection tube (provided).
- 11. Add $650 \mu l$ Buffer AW1 to the MB spin column. Centrifuge at $12,000 \times g$ for 1 min. Discard the flow-through and place the MB spin column back into the same 2 ml collection tube.
- 12. Add 650 μ l of Buffer AW2 to the MB spin column. Centrifuge at 12,000 x g for 1 min. Discard the flow-through and place the MB spin column into the same 2 ml collection tube.
- 13. Centrifuge at up to 16,000 x g for 2 min. Place the MB spin column into a new 1.5 ml elution tube (provided).
- 14. Add 50–100 µl of Buffer EB to the center of the white filter membrane.
- 15. Centrifuge at $12,000 \times g$ for 1 min. Discard the MB spin column. The DNA is now ready for downstream applications.

Revision History

Date	Changes
August 2019	Updated storage conditions. Rephrased homogenization instructions for TissueLyser 2, for clarity.

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

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