

Protocol for Isolation of DNA from Plant Tissue with the DNeasy Plant Mini Kit

Important notes before starting

- If using DNeasy Plant Mini Kits for the first time please read “Technical Information” (page 11).
- Buffers AP1 and AP3/E concentrate may form precipitates upon storage. If necessary, warm to 65°C to redissolve (before adding ethanol to Buffer AP3/E). Do not heat Buffer AP3/E after ethanol has been added
- Buffer AP1 may develop a yellow color upon storage. This does not affect the procedure.
- Buffers AW and AP3/E are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a water bath or heating block to 65°C.
- Preheat Buffer AE to 65°C.
- All centrifugation steps are carried out at room temperature in a microcentrifuge.

1. **Grind plant or fungal tissue under liquid nitrogen to a fine powder using a mortar and pestle. Transfer the tissue powder and liquid nitrogen to an appropriately sized tube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw. Continue immediately with step 2.**

Note: See “Disruption of plant material” (page 11).

2. **Add 400 µl of Buffer AP1 and 4 µl of RNase A stock solution (100 mg/ml) to a maximum of 100 mg of ground (wet weight) or 20 mg (dried) plant or fungal tissue and vortex vigorously.**

No tissue clumps should be visible. Vortex or pipet further to remove any clumps. Clumped tissue will not lyse properly and will therefore result in a lower yield of DNA. In the rare case where clumps cannot be removed by pipetting and vortexing, a disposable micropestle may be used.

Note: Do not mix Buffer AP1 and RNase A prior to use.

3. **Incubate the mixture for 10 min at 65°C. Mix 2–3 times during incubation by inverting tube.**

This step lyses the cells.

4. Add 130 μ l of Buffer AP2 to the lysate, mix, and incubate for 5 min on ice.

This step precipitates detergent, proteins, and polysaccharides.

(Optional) Centrifuge the lysate for 5 min at full speed.

Some plant materials can generate very viscous lysates and large amounts of precipitates during this step resulting in shearing of the DNA in the next step (see "Lysate filtration with QIAshredder", page 12). In this case optimal results are obtained if the majority of these precipitates are removed by centrifugation for 5 min at maximum speed. After centrifugation, apply supernatant to QIAshredder spin column and continue with step 5.

5. Apply the lysate to the QIAshredder spin column (lilac) sitting in a 2 ml collection tube and centrifuge for 2 min at maximum speed.

It may be necessary to cut the end off the pipette tip to apply the lysate to the QIAshredder column. QIAshredder removes most precipitates and cell debris, but a small amount will pass through and form a pellet in the collection tube. Be careful not to disturb this pellet in step 6.

6. Transfer flow-through fraction from step 5 to a new tube (not supplied) without disturbing the cell-debris pellet.

Typically 450 μ l of lysate is recovered. For some plant species less lysate is recovered. In this case determine volume for the next step.

7. Add 1.5 volumes of Buffer AP3/E to the cleared lysate and mix by pipetting.

Example: To 450 μ l lysate add 675 μ l Buffer AP3/E. Reduce the amount of Buffer AP3/E accordingly if less lysate is recovered. A precipitate may form after the addition of ethanol but this will not affect the DNeasy procedure.

Note: Ensure ethanol has been added to Buffer AP3/E (see "Important notes before starting").

Note: It is important to pipet Buffer AP3/E directly onto the cleared lysate and to mix immediately.

8. Apply 650 μ l of the mixture from step 7, including any precipitate which may have formed, to the DNeasy mini spin column sitting in a 2 ml collection tube (supplied). Centrifuge for 1 min at $\geq 6000 \times g$ (corresponds to ≥ 8000 rpm for most microcentrifuges) and discard flow-through.*

Reuse the collection tube in step 9.

9. Repeat step 8 with remaining sample. Discard flow-through* and collection tube.**10. Place DNeasy column in a new 2 ml collection tube (supplied), add 500 μ l Buffer AW to the DNeasy column and centrifuge for 1 min at $\geq 6000 \times g$ (≥ 8000 rpm). Discard flow-through and reuse the collection tube in step 11.**

Note: Ensure ethanol is added to Buffer AW (see page 15).

* Flow-through fractions contain Buffer AP3/E, and are therefore not compatible with bleach.

11. Add 500 μ l Buffer AW to the DNeasy column and centrifuge for 2 min at maximum speed to dry the membrane.

It is important to dry the membrane of the DNeasy column since residual ethanol may interfere with subsequent reactions. This spin ensures that no residual ethanol will be carried over during elution. Discard flow-through and collection tube.

After washing with Buffer AW, the DNeasy mini spin column membrane is usually only slightly colored. In the rare case that the membrane remains significantly colored after washing with Buffer AW, refer to “Darkly colored membrane” in the Troubleshooting Guide on page 21.

Note: Following the spin, remove the DNeasy column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

12. Transfer the DNeasy column to a 1.5 ml or 2 ml microcentrifuge tube (not supplied) and pipet 100 μ l of preheated (65°C) Buffer AE directly onto the DNeasy membrane. Incubate for 5 min at room temperature and then centrifuge for 1 min at $\geq 6000 \times g$ (≥ 8000 rpm) to elute.

Elution with 50 μ l (instead of 100 μ l) increases the final DNA concentration in the eluate significantly, but also reduces overall DNA yield. If larger amounts of DNA (>20 μ g) are loaded, eluting with 200 μ l (instead of 100 μ l) increases yield. See “Elution” on page 12.

13. Repeat elution (step 12) once as described.

A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the microcentrifuge tube can be reused for the second elution step to combine the eluates.

Note: More than 200 μ l should not be eluted into a 1.5 ml microcentrifuge tube because the DNeasy column will contact the eluate.