
Protocol: Purification of Total DNA from Plant Tissue (Mini Protocol)

Important points before starting

- If using the DNeasy Plant Mini Kit for the first time, read “Important Notes” (page 18).
- Ensure that you are familiar with operating the TissueRuptor II or the TissueLyser. See “Disruption and homogenization using the TissueRuptor II” or “Disruption and homogenization using the TissueLyser system”. Refer to the *TissueRuptor II User Manual* or the *TissueLyser Handbook* for operating instructions.
- Buffer AP1 may develop a yellow color upon storage. This does not affect the procedure.
- All centrifugation steps are carried out at room temperature in a microcentrifuge.

Things to do before starting

- Buffer AP1 and Buffer AW1 concentrate may form precipitates upon storage. If necessary, warm to 65°C to redissolve (before adding ethanol to Buffer AW1). Do not heat Buffer AW1 after ethanol has been added.
- Buffer AW2 and Buffer AW1 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.

Procedure

1. For disruption using the TissueRuptor II, follow step 2; for disruption using the TissueLyser, follow steps 3–6.

Alternatively, plant or fungal tissue can be ground to a fine powder under liquid nitrogen 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. Proceed immediately to step 7.

2. **TissueRuptor II procedure:** Place the sample material (≤ 100 mg wet weight or ≤ 20 mg lyophilized tissue) into a 2 ml microcentrifuge tube. Add liquid nitrogen to the tube, and freeze the sample for 30 s. Keep the sample submerged in liquid nitrogen, and disrupt for approximately 30 s at full speed. Allow the liquid nitrogen to evaporate, and proceed immediately to step 7.

Alternatively, fresh or lyophilized material can be directly disrupted in lysis buffer (after step 7) without using liquid nitrogen, but this may cause shearing of high-molecular-weight DNA. We do not recommend disrupting frozen material in lysis buffer, because this can result in low yields and degraded DNA.

3. **TissueLyser procedure:** Place the sample material (≤ 100 mg wet weight or ≤ 20 mg lyophilized tissue) into a 2 ml safe-lock microcentrifuge tube, together with a 3 mm tungsten carbide bead. Freeze the tubes in liquid nitrogen for 30 s.

When using lyophilized tissue, the tubes do not need to be frozen in liquid nitrogen.

4. Place the tubes into the TissueLyser Adapter Set 2 x 24, and fix into the clamps of the TissueLyser. Immediately grind the samples for 1 min at 30 Hz.
5. Disassemble the adaptor set, remove the tubes, and refreeze in liquid nitrogen for 30 s.
When using lyophilized tissue, the tubes do not need to be frozen in liquid nitrogen.
6. Repeat step 4, reversing the position of the tubes within the adaptor set. Proceed immediately to step 7.

Note: To prevent variation in sample homogenization, the adaptor sets should be removed from the TissueLyser and disassembled after the first disruption step. For the second disruption step, the adaptor sets should be reassembled so that the tube order is reversed. Rotating the racks of tubes in this way ensures that all samples are thoroughly and equally disrupted.

Note: The majority of plant tissue is ground to a fine powder after two disruption steps. However, for some materials, one disruption step may be sufficient. Other tissues, such as seeds and roots, may require three disruption steps. Optimization of the disruption procedure may be required for some plant material.

7. Add 400 μ l Buffer AP1 and 4 μ l RNase A stock solution (100 mg/ml) to a maximum of 100 mg (wet weight) or 20 mg (dried) disrupted plant or fungal tissue and vortex vigorously.

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

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

8. Incubate the mixture for 10 min at 65°C. Mix 2–3 times during incubation by inverting tube. This step lyses the cells.
9. Add 130 μ l Buffer P3 to the lysate. Mix and incubate for 5 min on ice.

This step precipitates detergent, proteins, and polysaccharides.

10. **Recommended:** Centrifuge the lysate for 5 min at 20,000 \times g (14,000 rpm).

Some plant materials can generate very viscous lysates and large amounts of precipitates during this step. This can result in shearing of the DNA in the next step (see “Lysate filtration with QIAshredder”, page 24). In this case, optimal results are obtained if the majority of these precipitates are removed by centrifugation for 5 min at 20,000 \times g (14,000 rpm). After centrifugation, apply supernatant to QIAshredder Mini spin column and continue with step 11.

11. Pipet the lysate into the QIAshredder Mini spin column (lilac) placed in a 2 ml collection tube, and centrifuge for 2 min at 20,000 \times g (14,000 rpm).

It may be necessary to cut the end off the pipette tip to apply the lysate to the QIAshredder Mini spin column. The QIAshredder Mini spin column 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 12.

12. Transfer the flow-through fraction from step 11 into a new tube (not supplied) without disturbing the cell-debris pellet.

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

13. Add 1.5 volumes of Buffer AW1 to the cleared lysate, and mix by pipetting.

For example, to 450 µl lysate, add 675 µl Buffer AW1. Reduce the amount of Buffer AW1 accordingly if the volume of lysate is smaller. A precipitate may form after the addition of Buffer AW1, but this will not affect the DNeasy procedure.

Note: Ensure that ethanol has been added to Buffer AW1.

Note: It is important to pipet Buffer AW1 directly onto the cleared lysate and to mix immediately.

14. Pipet 650 µl of the mixture from step 13, including any precipitate that may have formed, into the DNeasy Mini spin column placed 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 the flow-through.* Reuse the collection tube in step 15.
15. Repeat step 14 with remaining sample. Discard flow-through* and collection tube.
16. Place the DNeasy Mini spin column into a new 2 ml collection tube (supplied), add 500 µl Buffer AW2 and centrifuge for 1 min at $\geq 6000 \times g$ (≥ 8000 rpm). Discard the flow-through and reuse the collection tube in step 17.

Note: Ensure that ethanol is added to Buffer AW2.

* Flow-through fractions contain Buffer AW1 and are therefore not compatible with bleach. See "Safety Information".