Protocol
Plant + Fungi

RNeasy Plant Mini Protocol for Isolation of Total RNA from Plant Cells and Tissues, and Filamentous Fungi

Important notes before starting

- Total RNA isolation from plant cells and tissues and filamentous fungi requires the RNeasy Plant Mini Kit and cannot be performed with the RNeasy Mini Kit alone.
- A maximum of 100 mg plant material or 1 x 10^7 cells can be used per preparation. See “How much starting material can I use?” (page 12) for more information.
- If using RNeasy for the first time, please read “Disruption and homogenization of starting materials” (page 16).
- If preparing RNA for the first time, please read Appendix A (page 54).
- The RNeasy Plant Mini Kit provides two different lysis buffers, Buffer RLT and Buffer RLC, which contain guanidine isothiocyanate (GITC) or guanidine hydrochloride (GuHCl), respectively. In most cases Buffer RLT is the lysis buffer of choice due to the greater cell disruption and denaturation properties of GITC. However, depending on the amount and type of secondary metabolites in some tissues (such as milky endosperm of maize or mycelia of filamentous fungi), GITC can cause solidification of the sample, making extraction of RNA impossible. In these cases Buffer RLC should be used.
- Buffer RPE is supplied as a concentrate. Before using for the first time add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer RLT may form a precipitate upon storage. If necessary, warm to redissolve.
- β-Mercaptoethanol (β-ME) must be added to Buffer RLT or Buffer RLC before use (see page 20). Add 10 µl β-ME per 1 ml of Buffer RLT or Buffer RLC. The solution is stable for 1 month.
- The time between harvesting of tissue and freezing should be minimized. Once tissue is frozen, do not allow to thaw.
- After disruption, all steps of the RNeasy protocol should be performed at 20 to 25°C. During the procedure, work quickly.
- All centrifugation steps should be performed in a microcentrifuge at 20 to 25°C.
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1. Grind sample 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: Incomplete grinding of the starting material will lead to reduced RNA yields.

2. Add 450 µl of either Buffer RLT or Buffer RLC (see notes above) to a maximum of 100 mg of tissue powder. Vortex vigorously.

   A short (1–3 min) incubation at 56°C may help to disrupt tissue. However for samples with high starch content, incubation at elevated temperatures should be omitted to prevent swelling of the starting material.

   Note: Ensure bME is added to Buffer RLT or Buffer RLC before use (see “Important notes before starting”).

3. Apply lysate to the QIAshredder spin column (lilac) sitting in a 2-ml collection tube, and centrifuge for 2 min at maximum speed. Transfer flow-through fraction from QIAshredder to a new tube (not supplied) without disturbing the cell-debris pellet in the collection tube.

   It may be necessary to cut the end off the pipet tip to apply the lysate to the QIAshredder spin column. This centrifugation through QIAshredder removes cell debris and simultaneously homogenizes the lysate. While most of the cell debris is retained on the QIAshredder spin column, a very small amount of cell debris will pass through and form a pellet in the collection tube. Be careful not to disturb this pellet while transferring the lysate to a new tube (not supplied).

4. Add 0.5 volumes (usually 225 µl) ethanol (96–100%) to the cleared lysate and mix well by pipetting.

   If some lysate is lost during homogenization, reduce volume of ethanol proportionally. A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

5. Apply sample (usually 675 µl), including any precipitate which may have formed, onto an RNeasy mini spin column (pink) sitting in a 2-ml collection tube. Centrifuge for 15 sec at ≥8000 x g (≥10,000 rpm).

   If the volume of the mixture exceeds 700 µl, load aliquots successively onto the RNeasy column and centrifuge as above. Reuse the same collection tube but discard flow-through* after each step. Reuse the collection tube in step 6.

* Flow-through contains Buffer RLT and is therefore not compatible with bleach.
6. Pipet 700 µl Buffer RW1 onto the RNeasy column, and centrifuge for 15 sec at ≥8000 x g (≥10,000 rpm) to wash.
   Discard flow-through* and collection tube.

7. Transfer RNeasy column into a new 2-ml collection tube (supplied). Pipet 500 µl Buffer RPE onto the RNeasy column, and centrifuge for 15 sec at ≥8000 x g (≥10,000 rpm).
   Discard flow-through and reuse the collection tube in step 8.
   Note: Ensure ethanol is added to Buffer RPE before use (see “Important notes before starting”).

8. Add 500 µl Buffer RPE to the RNeasy column, and centrifuge for 2 min at maximum speed to dry the RNeasy membrane. Continue directly with step 9, or to eliminate any chance of possible Buffer RPE carryover, continue first with step 8a.
   It is important to dry the RNeasy membrane 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.
   Note: Following the spin, remove the RNeasy column from the collection tube carefully so that the column does not contact the flowthrough as this will result in carryover of ethanol.

8a. (Optional): Place the RNeasy spin column in a new 2-ml collection tube (not provided), and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

9. Transfer RNeasy column into a new 1.5-ml collection tube (supplied), and pipet 30–50 µl of RNase-free water directly onto the RNeasy membrane. Centrifuge for 1 min at ≥8000 x g (≥10,000 rpm) to elute. Repeat if the expected RNA yield is >20 µg.
   If a second elution step is performed, elute into the same collection tube using another 30–50 µl RNase-free water.

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach.