RNeasy® Plant Mini Kit

The RNeasy Plant Mini Kit (cat. nos. 74903 and 74904) can be stored at room temperature (15–25°C) for at least 9 months.

For more information, additional and more detailed protocols, and safety information, please refer to the *RNeasy Mini 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

- The RNeasy Plant Mini Kit provides a choice of lysis buffers. Buffer RLT is the lysis buffer of choice but Buffer RLT can cause solidification of some samples, depending on the amount and type of secondary metabolites in the tissue. In these cases, Buffer RLC should be used.
- Add either 10 µl β-mercaptoethanol (β-ME), or 20 µl 2 M dithiothreitol (DTT)*, to 1 ml Buffer RLT or Buffer RLC before use. Buffers with DTT or β-ME can be stored at room temperature for up to 1 month.
- Add 4 volumes of ethanol (96–100%) to Buffer RPE for a working solution.

* This option not included for plant tissue in handbook; handbook to be updated.

1. Disrupt a maximum of 100 mg plant material according to step 1a or 1b.

1a. Disruption with mortar and pestle
Immediately place tissue in liquid nitrogen. Grind thoroughly. Decant tissue powder and liquid nitrogen into RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw. Proceed immediately to step 2.

1b. Disruption using the TissueLyser II, TissueLyser LT, or TissueRuptor®
For detailed information on disruption of plant tissues for purification of RNA, see *TissueLyser Handbook*, *TissueLyser LT Handbook,* or *TissueRuptor Handbook*. (The *RNeasy Mini Handbook* will be updated with this option.)

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2. Add 450 µl Buffer RLT or Buffer RLC to a maximum of 100 mg tissue powder. Vortex vigorously.

3. Transfer the lysate to a QIAshredder spin column (lilac) placed in a 2 ml collection tube. Centrifuge for 2 min at full speed. Transfer the supernatant of the flow-through to a new microcentrifuge tube (not supplied) without disturbing the cell-debris pellet.

4. Add 0.5 volume of ethanol (96–100%) to the cleared lysate, and mix immediately by pipetting. Do not centrifuge. Proceed immediately to step 5.

5. Transfer the sample (usually 650 µl), with any precipitate, to an RNeasy Mini spin column (pink) in a 2 ml collection tube (supplied). Close the lid, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.

6. Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.

7. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 15 s at ≥8000 x *g*. Discard the flow-through.

8. Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 2 min at ≥8000 x *g*.

   Optional: Place the RNeasy spin column in a new 2 ml collection tube (supplied). Centrifuge at full speed for 1 min to dry the membrane.

9. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid, and centrifuge for 1 min at ≥8000 x *g* to elute the RNA.

10. If the expected RNA yield is >30 µg, repeat step 9 using another 30–50 µl of RNase-free water. Alternatively, use the eluate from step 9 (if high RNA concentration is required). Reuse the collection tube from step 9.