# 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 if not otherwise stated on label.

## 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. **Use RLT for leaf and RLC for endosperm**
* 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. **450 uL β-ME**
* Add 4 volumes of ethanol (96–100%) to Buffer RPE for a working solution. **44ml ethanal**
* **Cut tips of 24 (or whatever number of samples) 1000uL pipette tips**
* **Prepare 100% ethanal in 50ml tubes**

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.)

2. Add 450 μl Buffer RLT or Buffer RLC to a maximum of 100 mg tissue powder. Vortex vigorously. **Put sample tubes in liquid nitrogen before adding buffer**

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. **Use cut pipette tips**

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. **Add 225 uL ethanol to all samples and transfer**

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 700μl Buffer RW1 to the RNeasy spinc olumn. Close the lid, and centrifuge for 15s at ≥8000 x g (≥10,000 rpm). 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 (≥10,000 rpm). 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 (≥10,000 rpm).

**[skip]** 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 (≥10,000 rpm) to elute the RNA. **Discard old collection tube**

**[skip]** 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.