**GLOSSARY OF FANCY TERMS**

Lysis Buffer: Buffer solution used to break cells open

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](http://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](http://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 microliters Beta-mercaptoethanol (Beta-ME), or 20 microliters 2 M dithiothreitol (DTT)\*, to 1 ml Buffer RLT or Buffer RLC before use. Buffers with DTT or Beta-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 it while pretending it’s Ian (very thoroughly). Decant tissue powder and liquid nitrogen into RNase-free, liquid-nitrogen-cooled, 2 ml microcentrifuge tube (not supplied but we probably have?). 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 (WTF are those). 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 microliters 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, we probably have?) without disturbing the cell-debris pellet.

4. Add 0.5 volume of ethanol (units por favor) (96-100%) to the cleared lysate, and mix immediately by pipetting. DO NOT CENTRIFUGE. Go to step 5.

5. Transfer the sample (usually 650 microliters), with any precipitate, to an RNeasy Mini spin column (pink) in a 2 ml collection tube (supplied). Close the lid, and centrifuge dat boi for 15 seconds at >=8000 x g (>=10000 rpm). Discard the flow-through.

6. Add 700 microliter Buffer RW1 to the RNeasy spin column. Close the lid, and centrifuge for 15 seconds at >=8000 x g. Discard the flow-through.

7. Add 500 microliter Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 15 seconds at >=8000 x g. Discard the flow-through.

8. Add 500 microliter Buffer RPE to the RNeasy spin column. Close the lid, and centrifuge for 15 seconds at >=8000 x g. Discard the flow-through.

**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 microliter 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 microgram, repeat step 9 using another 30-50 microliter of RNase-free water. Alternatively, use the eluate from step 9 (if high RNA concentration is required). Reuse the collection tube from step 9.