User-Developed Protocol:

Isolation of total RNA from plant tissues using the RNeasy® Plant Mini Kit; alternative procedure

This procedure has been adapted from the RNeasy® Mini Protocol for Isolation of Total RNA from Plant Cells and Tissues and Filamentous Fungi, and is for use with the RNeasy Plant Mini Kit. The adapted procedure is based on McKenzie, D.J., McLean, M.A., Mukerji, S., and Green, M. (1997) Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription – polymerase chain reaction. Plant Disease **81**, 222. **It has not been thoroughly tested and optimized by QIAGEN**.

This protocol may be used for the isolation of RNA from plant tissues with high levels of phenolics and polysaccharides. It can also be used as an alternative to using Buffer RLC or RLT in the standard RNeasy Plant Protocol.

Please be sure to read the *RNeasy Mini Handbook* and the detailed RNeasy Mini Protocol for Isolation of Total RNA from Plant Cells and Tissues and Filamentous Fungi carefully before beginning this procedure.

Reagents and equipment to be supplied by user

- Lysis buffer:

  4 M guanidine isothiocyanate
  0.2 M sodium acetate, pH 5.0
  25 mM EDTA
  2.5% (w/v) PVP-40 (polyvinylpyrollidone, average molecular weight 40,000)
  1% (v/v) β-mercaptoethanol (β-ME),* add immediately before use

- 20% (w/v) sarkosyl
- Ethanol (96–100%)
- Liquid nitrogen†
- Mortar and pestle (alternatively: bead mill, e.g., Mixer Mill MM 300 from QIAGEN)
- Shaking water bath or heating block
- Microcentrifuge (with rotor for 2 ml tubes)
- Sterile, RNase-free tips

* *β-Mercaptoethanol is toxic; dispense in a fume hood and wear appropriate protective clothing.*

† *Liquid nitrogen can cause severe burns. Take appropriate safety measures.*

Procedure

1. **Grind sample (up to 50 mg) 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.

   Alternatively, plant tissue may be disrupted in a bead mill using steel or tungsten carbide beads. The plant material, beads, and disruption vessel must all be precooled in liquid nitrogen. Disruption is performed without lysis buffer.

2. **Add 600 µl Lysis buffer (see above for composition) to a maximum of 50 mg of tissue powder. Vortex vigorously.**

   Note: Ensure that β-ME is added to the Lysis buffer before use.

3. **Add 60 µl of 20% sarkosyl. Incubate at 70°C in a water bath or heating block for 10 min with vigorous shaking.**

   Note: For samples with a high starch content, incubation at elevated temperatures should be omitted to prevent swelling of the starting material.

4. **Pipet the lysate directly onto a QIAshredder™ Spin Column (lilac, supplied in the RNeasy Plant Mini Kit) placed in a 2 ml collection tube. Centrifuge for 2 min at maximum speed (14,000–18,000 x *g*). Carefully transfer the supernatant of the flow-through fraction to a new microcentrifuge 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 in order to pipet the lysate onto the QIAshredder Spin Column. Centrifugation through the QIAshredder Spin Column 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 microcentrifuge tube (not supplied).

5. **Add 0.5 volumes (usually 300 µl) ethanol (96–100%), and mix well by pipetting.**

   If some lysate is lost during homogenization (step 4), reduce volume of ethanol proportionally. A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

6. **Continue with step 6 of the RNeasy Mini Protocol for Isolation of Total RNA from Plant Cells and Tissues and Filamentous Fungi in the *RNeasy Mini Handbook*.**

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