User-Developed Protocol:

Isolation of total RNA from woody plant tissues using the RNeasy® Plant Mini Kit

This 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 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. **It has not been thoroughly tested and optimized by QIAGEN.**

The procedure has been used successfully for isolation of total and viral RNA from bark tissue, leaf tissue, and blossom tissue of woody plants.

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%)
- Homex homogenizer (BIOREBA, Carrboro, NC, USA)
- 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.*

Procedure

1. **Prepare shavings of woody tissue using, for example, a mechanical pencil sharpener. Proceed immediately with step 2.**

   Alternatively, wood shavings may be stored at –70°C for later use.

2. **Place a maximum of 600 mg tissue in a suitably sized sample bag for the homogenizer. Add 5 ml of room temperature Lysis buffer (see above for composition), and homogenize using a Homex homogenizer until the sample is uniformly homogeneous.**

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

3. **Transfer 1 ml of the homogenate to a microcentrifuge tube. Add 100 µl of 20% sarkosyl. Incubate at 70°C in a water bath or heating block for 10 min with vigorous shaking.**

4. **Pipet 650 µl of 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*).**

5. **Pipet 250 µl of the flow-through to a new 1.5 ml microcentrifuge tube. Add 225 µl ethanol (96–100%), and mix well by pipetting.**

   Discard remaining flow-through.* A precipitate may form after the addition of ethanol, but this will not affect the RNeasy procedure.

6. **Pipet sample, including any precipitate that may have formed, directly onto an RNeasy Mini Spin Column (pink, supplied in the RNeasy Plant Mini Kit) placed in a 2 ml collection tube. Centrifuge for 45 s at ≥8000 x *g* (≥10,000 rpm).**

   Discard flow-through,* and reuse the collection tube in the following wash step.

7. **Continue with step 7 of the RNeasy Mini Protocol for Isolation of Total RNA from Plant Cells and Tissues and Filamentous Fungi in the *RNeasy Mini Handbook*.**

* *Flow-through contains guanidine isothiocyanate, which is an irritant and not compatible with disinfecting agents containing bleach. Take appropriate safety measures and wear gloves when handling.*

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