User-Developed Protocol:

Purification of total RNA from plant latex using the RNeasy® Lipid Tissue Mini Kit

This protocol has been adapted by customers from the RNeasy Lipid Tissue Mini protocol and is intended as a guideline for the purification of total RNA from plant latex using the RNeasy Lipid Tissue Mini Kit. **This protocol has not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *RNeasy Lipid Tissue Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier. RNeasy Lipid Tissue Kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by user

- RNeasy Lipid Tissue Mini Kit (cat. no. 74804)
- Lysis buffer (4 M guanidinium isothiocyanate, 100 mM Tris·Cl, pH 7.0)
- Chloroform
- Ethanol (70%) (do not use denatured alcohol, which contains other substances such as methanol or methylethylketone)
- Sterile, RNase-free pipet tips
- Disposable gloves
- 1.5 ml or 2 ml microcentrifuge tubes
- Microcentrifuge(s) (with rotor for 2 ml tubes) for centrifugation at 4ºC and at room temperature (15–25ºC)
- Optional: RNase-Free DNase Set (cat. no. 79254)

Important points before starting

- If using RNeasy Lipid Tissue Kits for the first time, read "Important Notes" (page 10) in the *RNeasy Lipid Tissue Handbook*.
- If working with RNA for the first time, read Appendix A (page 28) in the *RNeasy Lipid Tissue Handbook*.
- Generally, DNase digestion is not required since integrated QIAzol and RNeasy technologies efficiently remove most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., real-time RT-PCR analysis of a low-abundance target). In these cases, residual DNA can be removed by optional on-column DNase-digestion using the RNase-Free DNase Set (see Appendix C, page 32, in the *RNeasy Lipid Tissue Handbook*). Alternatively, for real-time two-step RT-PCR applications, the QuantiTect® Reverse Transcription Kit provides cDNA synthesis with integrated removal of genomic DNA contamination.

- QIAzol Lysis Reagent and Buffer RW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 6 in the *RNeasy Lipid Tissue Handbook* for safety information.
- Except for phase separation (step 6), all protocol and centrifugation steps should be performed at room temperature (15–25ºC). During the procedure, work quickly.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- If performing optional on-column DNase digestion, prepare DNase I stock solution as described in Appendix C (page 32) in the *RNeasy Lipid Tissue Handbook*.

Procedure

1. **Add 50 µl plant latex to 100 µl lysis buffer (4 M guanidinium isothiocyanate, 100 mM Tris·Cl, pH 7.0) in a 2 ml microcentrifuge tube.**
   The lysis buffer can be omitted, but this may lead to a reduction in RNA yield and quality.
2. **Add 900 µl QIAzol Lysis Reagent to the tube.**
   If lysis buffer was omitted in step 1, add a total of 950 µl QIAzol Lysis Reagent to the tube.
3. **Place the tube on the benchtop at room temperature (15–25ºC) for 5 min.**
   This step promotes dissociation of nucleoprotein complexes.
4. **Add 200 µl chloroform to the tube. Securely cap the tube, and vortex vigorously.**
   Thorough mixing is important for subsequent phase separation.
5. **Place the tube on the benchtop at room temperature for 2–3 min.**
6. **Centrifuge at 12,000 x *g* for 15 min at 4ºC. After centrifugation, heat the centrifuge to room temperature (15–25ºC) if the same centrifuge will be used in the later steps of this procedure.**
   After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. The volume of the aqueous phase should be approximately 500 µl.
7. **Transfer the upper, aqueous phase to a new tube (not supplied). Add 1 volume (usually 500 µl) of 70% ethanol, and mix thoroughly by vortexing. Do not centrifuge. Proceed immediately to step 8.**
   Note: The volume of lysate may be less than 500 µl due to loss during centrifugation.
   Precipitates may be visible after addition of ethanol, but do not affect RNA purification.
8. **Transfer up to 700 µl of the sample to an RNeasy Mini spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) at room temperature (15–25ºC). Discard the flow-through.***
   Reuse the collection tube in step 9.
9. **Repeat step 8 using the remainder of the sample. Discard the flow-through.***
   Reuse the collection tube in step 10.

* Flow-through contains QIAzol Lysis Reagent and is therefore not compatible with bleach. See page 6 in the *RNeasy Lipid Tissue Handbook* for safety information.

Optional: If performing optional on-column DNase digestion (see “Important points before starting”), follow steps C1–C4 (page 32) in the *RNeasy Lipid Tissue Handbook* after performing this step, and then proceed to step 11.

10. **Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the membrane. Discard the flow-through.***

    Reuse the collection tube in step 11.

    After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.*

    Note: Skip this step if performing optional on-column DNase digestion.

11. **Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the membrane. Discard the flow-through.**

    Reuse the collection tube in step 12.

    Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see “Things to do before starting”).

12. **Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the membrane.**

    The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.

    Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

13. **Recommended: Place the RNeasy spin column in a new 2 ml collection tube (supplied), and discard the old collection tube with the flow-through. Close the lid gently, and centrifuge at full speed for 1 min.**

    Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column after step 12.

14. **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 gently. To elute the RNA, centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm).**

15. **If the expected RNA yield is >30 µg, repeat step 14 using another volume of RNase-free water, or using the eluate from step 14 (if high RNA concentration is required). Reuse the collection tube from step 14.**

    If using the eluate from step 14, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 6 in the RNeasy Lipid Tissue Handbook for safety information.

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