QIAGEN Supplementary Protocol:

Isolation of total RNA from plant tissue using the QIAGEN-tip

This protocol is designed for isolation of up to 200 µg RNA from 150 mg plant tissue or up to 1 mg RNA from 600 mg plant tissue and is for use with QIAGEN-tip 100 or QIAGEN-tip 500, respectively.

Please be sure to read the *QIAGEN® Plasmid Purification Handbook*, *QIAGEN Genomic DNA Handbook*, or *QIAGEN RNA/DNA Handbook* and the detailed QIAGEN-tip Protocol carefully before beginning this procedure. If preparing RNA for the first time, please read either the *RNeasy® Mini Handbook* or *QIAGEN RNA/DNA Handbook* for general information about handling RNA.

Single-underlined text denotes QIAGEN-tip 100.

Double-underlined text denotes QIAGEN-tip 500.

Important notes before starting

- The yield of RNA is very tissue-specific and should be optimized for the amount of tissue used. Adjust the amount of tissue accordingly so as not to exceed the RNA-binding capacity of the QIAGEN-tip (200 µg or 1000 µg). Buffer volumes in steps 1–5 may be scaled linearly to any reasonable volume.
- It is very important not to overload the QIAGEN-tip. Overloading will significantly reduce yield and purity.
- Prepare Buffers R1, R4, R5, R6, QAT, QA, and QR. See "Composition of buffers" below.
- A working solution of Buffer QRU must be prepared by dissolving urea in Buffer QR just prior to use. Check pH before use, and adjust to 7.0 if necessary. See "Composition of Buffers" below.
- Other reagents required are β-mercaptoethanol, 25% Triton® X-100, isopropanol, 80% ethanol, and sterile water or buffer for dissolving the purified RNA.

Procedure

1. **For 150 mg or 600 mg tissue, add 3 ml or 9 ml ice-cold Buffer R1 and 3 µl or 9 µl β-mercaptoethanol. Homogenize with an Ultra Turrax® homogenizer (setting 7) for 3 to 5 intervals of 15 s each.**

   Alternative methods of homogenization or different buffer volumes may be used, but the ratio of tissue to buffer volume should not be altered. The volume of homogenization Buffer R1 in ml should be about 20 times the mass of the tissue in g (i.e., a 5% w/v homogenate). If using volumes different from those given here, be sure to scale buffer volumes in steps 1–5 accordingly.

   Note: β-mercaptoethanol is toxic; dispense in a fume hood and wear appropriate protective clothing.

2. **Add 240 µl or 720 µl of 25% Triton X-100. Mix well, and incubate on ice for 15 min.**
3. **Add 3 ml or 9 ml ice-cold Buffer R4. Mix well, and incubate on ice for another 15 min.**

4. **Centrifuge at 15,000 x *g* for 30 min at 4°C.**
   This step removes insoluble contaminants.

5. **Carefully decant the supernatant into a clean centrifuge tube. Add 0.8 volumes isopropanol, and incubate on ice for 5 min to precipitate RNA.**

6. **Centrifuge at 15,000 x *g* for 30 min at 4°C. Carefully remove and discard the supernatant.**

7. **Dissolve the pellet in 8 ml or 16 ml ice-cold Buffer R5.**
   The pellet varies in size and consistency depending on the tissue used. Use a pipet with a cut-off tip to resuspend the pellet.

8. **If the suspension still contains particulate matter, centrifuge at 20,000 x *g* for 15 min at 4°C. Carefully decant the supernatant into a clean tube, and discard the pellet.**
   This step helps prevent subsequent clogging of the QIAGEN-tip.

9. **Add 2 ml or 4 ml Buffer R6 and mix well. Check the pH and adjust to 7.0 if necessary.**

10. **Equilibrate a QIAGEN-tip 100 or a QIAGEN-tip 500 with 3 ml or 10 ml Buffer QAT, and allow it to empty by gravity flow. Do not force out the remaining buffer.**
    Flow begins automatically by reduction in surface tension due to the presence of detergent (Triton X-100) in Buffer QAT.

11. **Apply the sample from step 9 to the equilibrated QIAGEN-tip. Allow it to enter the resin by gravity flow.**

12. **Wash the QIAGEN-tip with 15 ml or 30 ml Buffer QA.**

13. **Elute the RNA with 10 ml or 20 ml Buffer QRU.**
    Note: A working solution of Buffer QRU must be prepared by dissolving urea in Buffer QR just prior to use. See "Composition of buffers" below.

14. **Add 10 ml or 20 ml (1 volume) isopropanol to the eluted RNA, and incubate on ice for 10 min to precipitate the RNA. Centrifuge at 15,000 x *g* for 30 min at 4°C. Carefully remove the supernatant.**

15. **Wash the RNA pellet with 80% ethanol. Air-dry for 5 min, and dissolve the RNA in a suitable volume of sterile buffer or sterile water.**

Composition of buffers

Buffer	Composition	Storage
Buffer R1*	4 M guanidine thiocyanate (GITC), 100 mM Tris·Cl, 25 mM $MgCl_2$, 25 mM EDTA, pH 7.5	2–8°C
Buffer R4	3 M Na-acetate, pH 6.0	2–8°C
Buffer R5	20 mM Tris·Cl, 1 mM EDTA, pH 8.0	2–8°C
Buffer R6	2 M NaCl, 250 mM MOPS, pH 7.0	room temp.
Buffer QAT	400 mM NaCl, 50 mM MOPS, 15% ethanol, 0.15% Triton X-100, pH 7.0	room temp.
Buffer QA	400 mM NaCl, 50 mM MOPS, 15% ethanol, pH 7.0	room temp.
Buffer QR	1.2 M NaCl, 67 mM MOPS, 20% ethanol, pH 6.7	room temp.
Buffer QRU	Just prior to use, add 3.62 g or 7.23 g urea to 7.5 ml or 15 ml Buffer QR. The final volume is 10 ml or 20 ml. Check pH before use, and adjust to 7.0 if necessary.	prepare fresh

* *Not compatible with disinfection reagents containing bleach. Contains guanidine thiocyanate, which is an irritant. Take appropriate safety measures, and wear gloves when handling.*

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Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from **www.qiagen.com/ts/msds.**