

Isolation of total RNA, including small RNAs, from plant tissues

A generic protocol for RNA extraction from plants is difficult to suggest, as plants differ widely in their physical makeup and can contain large amounts of problematic material which can interfere with downstream analysis. The protocol below should be sufficient for simple green leaves, soft roots, and other plant parts. We recommend a literature search for publications detailing more specialized isolation procedures for your specific sample type; alternatively, call QIAGEN technical support for assistance.

Equipment and reagents to be supplied by user

- miRNeasy Mini Kit (cat. no. 217004)
- TissueLyser II (cat. no. 85300)
- TissueLyser Adapter Set 2 x 24 (cat. no. 69982)
- Stainless Steel Beads, 5 mm (cat. no. 69989)
- MaXtract™ High Density Tubes, 2 ml (cat. no. 129056)
- Chloroform (without added isoamyl alcohol)
- Ethanol (70% and 96–100%); do not use denatured alcohol, which contains other substances such as methanol and methylethylketone
- Sterile, RNase-free pipet tips
- 1.5 ml or 2 ml microcentrifuge tubes
- Microcentrifuge(s) (with rotor for 2 ml tubes) for centrifugation at 4°C and at room temperature (15–25°C)
- Optional: RNase-Free DNase Set (cat. nos. 79254, 79256)

Important points before starting

- If using the miRNeasy Mini Kit for the first time, read “Important Notes” in the *miRNeasy Mini Handbook*.
- The TissueLyser II is recommended for optimal sample disruption and homogenization. As an alternative to the TissueLyser II, the TissueLyser LT or TissueRuptor® can be used. For more information, visit www.qiagen.com.
- This protocol is for isolation of total RNA, including miRNA, from fresh plant tissues.
- Buffer RWT may form a precipitate upon storage. If necessary, redissolve by warming and then place at room temperature (15–25°C).
- QIAzol® Lysis Reagent and Buffer RWT contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See the “Safety Information” section in the *miRNeasy Mini Handbook*. Except for phase separation (step 11), all protocol and centrifugation steps should be performed at room temperature.

Things to do before starting

Buffers RWT and RPE are supplied as concentrates. Before using for the first time, add the required volumes of ethanol (96–100%), as indicated on the bottle, to obtain a working solution.

Procedure

1. Transfer a single TissueLyser stainless steel bead to a 2 ml centrifuge tube.
2. Place 25 mg fresh plant tissue in the 2 ml centrifuge tube containing the stainless steel bead.
Note: Up to 50 mg plant tissue can be added. If there is a need to use 100 mg, perform separate 50 mg isolations and combine at the aqueous phases.
3. Add 1 ml QIAzol Lysis Reagent and securely cap the tube(s).
4. Place the tube(s) in the TissueLyser Adapter Set 2 x 24.
5. Operate the TissueLyser for 1 min at 20–30 Hz. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser are now outermost, and reassemble the adapter set. Operate the TissueLyser for another 1 min at 20–30 Hz. The duration of disruption and homogenization depends on the tissue being processed and can be extended until no tissue debris is visible.
6. Place the tube on the benchtop at room temperature (15–25°C) for 5 min. This step promotes dissociation of nucleoprotein complexes.
7. Immediately before use, pellet MaXtract High Density by centrifugation for 20–30 s at 12,000–16,000 x g in a microcentrifuge.

8. Transfer homogenized lysate (from step 6) to the MaXtract High Density tube.
9. To the MaXtract High Density Tube, add 200 μ l chloroform. Securely cap the tube and shake the tube vigorously for 15 s. Thorough mixing is important for subsequent phase separation.

IMPORTANT: Do not vortex.

10. Place the MaXtract High Density Tube on the benchtop at room temperature for 2–3 min.
11. Centrifuge for 15 min at 12,000 $\times$ g at 4°C. After centrifugation, heat the centrifuge to room temperature if the same centrifuge will be used for the next centrifugation steps. After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase (MaXtract High Density gel); and a lower, red, organic phase.
12. Transfer upper aqueous phase to a new tube. Add 1.5 volumes of 100% ethanol and mix thoroughly by pipetting up and down several times. Do not centrifuge. Continue without delay with Step 13.
13. Pipet up to 700 μ l of the sample into an RNeasy Mini spin column in a 2 ml collection tube. Close the lid gently and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) at room temperature. Discard the flow-through. * Reuse the collection tube in step 14.
14. Repeat step 13 using the remainder of the sample. Discard the flow-through. * Reuse the collection tube in step 15.

Optional: If performing optional on-column DNase digestion, follow steps B1–B4 (*miRNeasy Mini Kit Handbook*, page 36) after performing this step.

15. Add 700 μ l Buffer RWT into the RNeasy Mini Spin column and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash. Discard the flow-through. * Reuse the collection tube in step 16.
16. Pipet 500 μ l Buffer RPE into the column. Close lid and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the column. Discard flow-through. Reuse the collection tube in step 17.
17. Add another 500 μ l Buffer RPE to the column. Close the lid gently and centrifuge for 2 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to dry the RNeasy Mini spin column 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: Following centrifugation, remove the RNeasy Mini spin column from the collection tube carefully so the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

* Flow-through contains QIAzol Lysis Reagent and is therefore not compatible with bleach. See page 5 in the *miRNeasy Mini Kit Handbook* for safety information.

18. **Optional:** Place the RNeasy Mini spin column into a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge 1 min at full speed. Perform this step to eliminate any possible carryover of Buffer RPE or if residual flow-through remains on the outside of the RNeasy Mini spin column after step 17.
19. Transfer the RNeasy Mini spin column to a new 1.5 ml collection tube. Add 30 µl RNase-free water directly onto the RNeasy Mini spin column membrane. Close lid gently and centrifuge for 1 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to elute RNA.

If the expected yield is >30 µg, repeat Step 19 with a second volume of 20 µl RNase-free water. Elute into the same collection tube.

Note: To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate (from Step 19). The yield will be 15–30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

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