User-Developed Protocol

Purification of total RNA, including small RNAs, using the RNeasy® Midi Kit

This protocol is for the purification of total RNA, including small RNAs such as miRNA, from cells and tissues using the RNeasy Midi Kit. The spin columns supplied with the kit have a binding capacity of 1 mg RNA. This allows RNA purification from larger samples compared with the miRNeasy Mini Kit, which is supplied with spin columns with a binding capacity of 100 µg RNA. This procedure has been adapted by customers and is for use with the RNeasy Midi Kit, QIAzol® Lysis Reagent, and Buffer RWT: **It has not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please consult the "Safety Information" and "Important Notes" sections in the *RNeasy Midi/Maxi Handbook* and *QIAzol 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.

Equipment and reagents

- RNeasy Midi Kit (cat. no. 75142 or 75144)
- QIAzol Lysis Reagent (200 ml) (cat. no. 79306)
- Buffer RWT (concentrate, 80 ml) (cat. no. 1038708)
- TissueRuptor, TissueLyser, or other instrument for sample disruption and homogenization
- Chloroform
- 100% ethanol
- Refrigerated centrifuge capable of centrifuging 15 ml tubes at 3000–5000 x *g*

Important points before starting

- RNeasy Midi spin columns have a maximum binding capacity of 1 mg RNA.
- RNA can be purified from about 20 mg tissue up to a maximum of 250 mg tissue (or 40–500 mg brain or adipose tissue).
- RNA can be purified from about 5 x 10^6 cells up to a maximum of 1 x 10^8 cells. Note that certain cell lines (e.g., COS and HeLa cells) contain more RNA than others (e.g., NIH/3T3 cells). Therefore, when purifying from RNA-rich cell lines, use less than 1 x 10^8 cells as starting material.

Things to do before starting

- Buffer RWT is supplied as a concentrate. Before using for the first time, add 2 volumes of ethanol (96%–100%) as indicated on the bottle to obtain a working solution.
- 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.

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Procedure

1. **Add the cell or tissue sample to 5 ml QIAzol Lysis Reagent in an appropriately sized tube (not supplied).**
2. **Homogenize immediately with the TissueRuptor, TissueLyser, or other homogenizer.**
   This step is required for tissue samples, but is not necessary for cultured-cell samples.
3. **Place the tube on the benchtop at room temperature (15–25°C) for 5 min.**
4. **Add 1 ml chloroform. Cap the tube and shake by hand for 15 s.**
5. **Place the tube on the benchtop at room temperature (15–25°C) for 2–3 min.**
6. **Centrifuge the tube at 5000 x *g* for 15 min at 4°C.**
   After centrifugation, increase the temperature of the centrifuge to 20–25°C for steps 9–14.
7. **Transfer the upper aqueous phase to a new tube (not supplied).**
8. **Add 1.5 volumes of 100% ethanol, and mix thoroughly by vortexing.**
9. **Transfer up to 4 ml sample, including any precipitate, to an RNeasy Midi spin column placed in a 15 ml collection tube (supplied). Close the lid, and centrifuge at 5000 x *g* for 5 min at 20–25°C. Discard the flow-through.**
   If the volume of sample is greater than 4 ml, repeat step 9 until the entire sample has passed through the column.
10. **Add 4 ml Buffer RWT to the RNeasy Midi spin column. Close the lid, and centrifuge at 3000–5000 x *g* for 5 min at 20–25°C to wash the membrane. Discard the flow-through.**
11. **Add 2.5 ml Buffer RPE to the RNeasy Midi spin column. Close the lid, and centrifuge at 3000–5000 x *g* for 5 min at 20–25°C. Discard the flow-through.**
12. **Add 2.5 ml Buffer RPE to the RNeasy Midi spin column. Close the lid, and centrifuge at 3000–5000 x *g* for 5 min at 20–25°C. Discard the flow-through.**
13. **Place the RNeasy Midi spin column into a new 15 ml tube (not supplied) or reuse the collection tube from step 12. Open the lid, and centrifuge at 3000–5000 x *g* for 5 min at 20–25°C.**
    This step ensures removal of residual wash buffers in the membrane.
14. **Place the RNeasy Midi spin column into a new 15 ml collection tube (supplied). Add either 150 µl RNase-free water (if the expected RNA yield is ≤150 µg) or 250 µl RNase-free water (if the expected RNA yield is 150–1000 µg). Wait for 1 min, and centrifuge at 3000–5000 x *g* for 3 min at 20–25°C to elute the RNA.**
    Use the RNase-free water supplied with the kit.

    Optional: For maximum RNA yield, repeat the elution step using either the first eluate (if concentrated RNA is required) or another volume of RNase-free water.