User-Developed Protocol

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

This protocol is for the purification of total RNA, including small RNAs such as miRNA, from cells and tissues using the RNeasy Maxi Kit. The spin columns supplied with the kit have a binding capacity of 6 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 Maxi 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 Maxi Kit (cat. no. 75162)
- 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 50 ml tubes at 3000–5000 x *g*

Important points before starting

- RNeasy Maxi spin columns have a maximum binding capacity of 6 mg RNA.
- RNA can be purified from about 250 mg tissue up to a maximum of 1000 mg tissue (or 0.5–2 g brain or adipose tissue).
- RNA can be purified from about 5×10^7 cells up to a maximum of 5×10^8 cells.

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.

Procedure

1. **Add the cell or tissue sample to 10 ml QIAzol Lysis Reagent (i.e., 1 ml reagent per 100 mg tissue) 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 2 ml chloroform (i.e., 0.2 ml chloroform per 1 ml QIAzol Lysis Reagent). 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 15 ml sample, including any precipitate, to an RNeasy Maxi spin column placed in a 50 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 15 ml, repeat step 9 until the entire sample has passed through the column.
10. **Add 15 ml Buffer RWT to the RNeasy Maxi spin column. Close the lid, and centrifuge at 3000–5000 x *g* for 5–10 min at 20–25°C to wash the membrane. Discard the flow-through.**
11. **Add 10 ml Buffer RPE to the RNeasy Maxi 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 10 ml Buffer RPE to the RNeasy Maxi 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 Maxi spin column into a new 50 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 Maxi spin column into a new 50 ml collection tube (supplied). Add either 0.8 ml RNase-free water (if the expected RNA yield ≤1 mg) or 1.2 ml RNase-free water (if the expected RNA yield is 1–6 mg). 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.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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

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QIAzol Lysis Reagent is a subject of US Patent No. 5,346,994 and foreign equivalents.

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