

QIAGEN Supplementary Protocol

Purification of miRNA from cells and tissues using the AllPrep[®] DNA/RNA/Protein Mini Kit and RNeasy[®] MinElute[®] Cleanup Kit

This protocol describes how to purify miRNA using the AllPrep DNA/RNA/Protein Mini Kit in combination with the RNeasy MinElute Cleanup Kit. miRNA is purified from cultured cells and harvested tissues of animal and human origin. By following the procedures in this protocol and the *AllPrep DNA/RNA/Protein Mini Handbook*, simultaneous purification of genomic DNA, total RNA (>200 nucleotides), miRNA, and total protein can be achieved.

IMPORTANT: Please consult the “Safety Information” and “Important Notes” sections in the *AllPrep DNA/RNA/Protein Mini Handbook* and *RNeasy MinElute Cleanup 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

- AllPrep DNA/RNA/Protein Mini Kit (cat. no. 80004)
- RNeasy MinElute Cleanup Kit (cat. no. 74204)
- Ethanol (100%, 96–100%, and 80%) (do not use denatured alcohol, which contains other substances such as methanol or methylethylketone)
- Optional: Extension Tubes (3 ml) (cat. no. 19587) and a QIAGEN[®] vacuum manifold

Important points before starting

- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- If using a refrigerated microcentrifuge, make sure cooling is switched off (i.e., set the temperature to just above ambient temperature). Make sure the microcentrifuge is at room temperature (15–25°C).

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.



Procedure

Follow the cell protocol (page 18) or tissue protocol (page 27) in the *AllPrep DNA/RNA/Protein Mini Handbook* up to and including step 14. Then follow steps 1–7 below to purify a small RNA fraction, which contains miRNA as well as other small RNAs such as tRNA. To purify total protein and genomic DNA, continue with steps 15–24 in the handbook.

1. **Centrifuge at full speed for 10 min, and carefully decant the supernatant. Transfer the supernatant to a new 5 ml tube (not supplied).**
2. **Add 1 volume of 100% ethanol to the supernatant, and mix well by pipetting up and down.**

Addition of ethanol adjusts the binding conditions for small RNAs.

3. **Transfer up to 700 μ l of the sample to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm). Discard the flow-through.* Repeat this step until the entire sample has passed through the RNeasy MinElute membrane.**

Optional: Alternatively, pass the entire sample through the membrane in a single step by placing the spin column on top of a 3 ml extension tube (cat. no. 19587) attached to a QIAGEN vacuum manifold and applying vacuum.

4. **Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Add 500 μ l Buffer RPE to the spin column. Close the lid gently, and centrifuge for 15 s at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane. Discard the flow-through.**

Reuse the collection tube in step 5.

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

5. **Add 500 μ l of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at $\geq 8000 \times g$ ($\geq 10,000$ rpm) to wash the spin column membrane. Discard the flow-through and collection tube.**

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

* Sample contains Buffer RLT and is therefore not compatible with bleach. See the *AllPrep DNA/RNA/Protein Mini Handbook* for safety information.

- 6. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the flow-through and collection tube.**

To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

It is important to dry the spin column membrane since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

- 7. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 μ l RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.**

As little as 10 μ l RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with less than 10 μ l RNase-free water as the spin column membrane will not be sufficiently hydrated.

The dead volume of the RNeasy MinElute spin column is 2 μ l: elution with 14 μ l RNase-free water results in a 12 μ l eluate.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature.

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

The AllPrep DNA/RNA/Protein Mini Kit and RNeasy MinElute Cleanup Kit are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products. Therefore, the performance characteristics of the products for clinical use (i.e., diagnostic, prognostic, therapeutic, or blood banking) are unknown.

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