User-Developed Protocol:

Purification of miRNA from total RNA, cultured cells, soft tissues, or plant tissues using the RNeasy® Mini Kit and RNeasy MinElute® Cleanup Kit

This user-developed protocol has been adapted by customers for the purification of miRNA from total RNA, cultured cells, soft tissues, or plant tissues using the RNeasy Mini Kit and the RNeasy MinElute Cleanup Kit. This user-developed protocol contains two alternative protocols: follow Protocol 1 if you want to co-purify miRNA with total RNA, or follow Protocol 2 if you want to purify miRNA and larger RNAs (>200 nt) separately. Protocol 2 can also be used to purify miRNA from crude preparations of total RNA. **These protocols have not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please be sure to read the *RNeasy Mini Handbook* and the *RNeasy MinElute Cleanup Handbook* before starting, paying careful attention to the Safety Information section.

miRNA Purification Procedures

Protocol 1: Co-purification of miRNA and total RNA

Disrupt and homogenize sample in Buffer RLT

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Add 3.5 volumes of 100% ethanol

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Apply to RNeasy Mini spin column

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Wash the spin column membrane

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Elute miRNA and total RNA

Protocol 2: Separate purification of miRNA and total RNA

Disrupt and homogenize sample in Buffer RLT

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Add 1 volume of 80% ethanol

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Apply to RNeasy Mini spin column

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Add 1.4 volumes of 100% ethanol to the flow-through

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Apply to RNeasy MinElute spin column

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Wash the spin column membrane

↓

Elute miRNA

Wash the spin column membrane

↓

Elute total RNA (>200 nucleotides)

User-developed protocol

Protocol 1: Copurification of miRNA and total RNA from cultured cells, soft tissues, or plant tissues using the RNeasy Mini Kit

Reagents and equipment to be supplied by user

- RNeasy Mini Kit (50) (cat. no. 74104)
- Ethanol (100%)

Important points before starting

- This protocol is for use with the following amounts of starting material:
  - 5 x 10^6 cultured cells, or
  - Up to 20 mg soft tissue, or
  - Up to 50 mg plant tissue
- All centrifugation steps are carried out at room temperature (15–25°C).
- Read the handbook supplied with the RNeasy Mini Kit.

Procedure

1. **Add 350 µl Buffer RLT to the sample, and disrupt and homogenize immediately.**

   For details on disrupting and homogenizing samples, refer to the *RNeasy Mini Handbook*.

2. **Add 3.5 volumes of 100% ethanol (1225 µl), and mix thoroughly by vortexing. Do not centrifuge. Proceed immediately to step 3.**

3. **Pipet 700 µl of the sample, including any precipitate that may have formed, into an RNeasy Mini spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.**

   Repeat step 3 until the whole sample has been pipetted into the spin column. Discard the flow-through each time.

4. **Place the RNeasy Mini spin column into a new 2 ml collection tube. Pipet 500 µl Buffer RPE into the spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**

   Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting" in the handbook supplied with the RNeasy Mini Kit).

5. **Pipet another 500 µl Buffer RPE into the RNeasy Mini spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and the collection tube.**

6. **Place the RNeasy Mini spin column into a new 2 ml collection tube. Centrifuge at full speed for 1 min.**

7. **Place the RNeasy Mini spin column into a 1.5 ml collection tube. Pipet 30–50 µl RNase-free water directly onto the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the miRNA and total RNA.**

   If the expected RNA yield is >30 µg, repeat step 7 with a second volume of RNase-free water. Elute into the same collection tube.

Protocol 2: Separate purification of miRNA and larger RNAs (>200 nt) from total RNA, cultured cells, soft tissues, or plant tissues using the RNeasy Mini Kit and RNeasy MinElute Cleanup Kit

Reagents and equipment to be supplied by user

- RNeasy Mini Kit (50) (cat. no. 74104)
- RNeasy MinElute Cleanup Kit (50) (cat. no. 74204)
- Ethanol (80% and 100%)
- Optional (only required for separate purification of large RNAs [>200 nt]): RNase-Free DNase Set (50) (cat. no. 79254)
- Collection tubes (2 ml and 1.5 ml) are supplied with both RNeasy Kits; if necessary, extra 2 ml collection tubes can be purchased separately (cat. no. 19201)

Important points before starting

- This protocol is for use with the following amounts of starting material:
  - 5×10^6 cultured cells, or
  - Up to 20 mg soft tissue, or
  - Up to 50 mg plant tissue, or
  - Up to 100 µg total RNA in a maximum volume of 50 µl (Total RNA purified using a phenol–chloroform-based method can be used, since it still contains miRNA. However, total RNA purified using an RNeasy Kit cannot be used, since it contains no miRNA.)
- All centrifugation steps are carried out at room temperature (15–25°C).

Things to do before starting

- Read the handbooks supplied with the RNeasy Mini Kit and the RNeasy MinElute Cleanup Kit.
- 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.
- Optional (only required for separate purification of large RNAs [>200 nt]): Prepare DNase I stock solution before using the RNase-free DNase for the first time. Dissolve the solid DNase I (1500 Kunitz units) in 550 µl of RNase-free water provided. Take care that no DNase I is lost when opening the vial. Mix gently by inverting the tube. **Do not vortex.**

  For long-term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

Separating miRNA from total RNA using the RNeasy Mini Kit

1. **Add 350 µl Buffer RLT to the sample, and disrupt and homogenize immediately.**

   For details on disrupting and homogenizing samples, refer to the *RNeasy Mini Handbook*.

2. **Add 1 volume of 80% ethanol (350 µl), and mix thoroughly by vortexing. Do not centrifuge. Proceed immediately to step 3.**
3. **Pipet the sample, including any precipitate that may have formed, into an RNeasy Mini spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm).**
4. **If purifying miRNA only, discard the RNeasy Mini spin column and follow steps 5 to 11 only.**

   If purifying both miRNA and total RNA, save the RNeasy Mini spin column for use in step 12 (the spin column can be stored at 4°C or at room temperature [15–25°C], but not for long periods). Follow steps 5 to 11 to purify miRNA, and then steps 12 to 19 to purify total RNA.

Purifying miRNA using the RNeasy MinElute Cleanup Kit

5. **Pipet the flow-through from step 3 (which contains miRNA) into a 2 ml reaction tube (not supplied).**
6. **Add 1.4 volumes of 100% ethanol (980 µl), and mix thoroughly by vortexing. Do not centrifuge. Proceed immediately to step 7.**
7. **Pipet 700 µl of the sample into an RNeasy MinElute spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.**

   Repeat step 7 until the whole sample has been pipetted into the spin column. Discard the flow-through each time.

8. **Pipet 500 µl Buffer RPE into the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.**

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

9. **Pipet 500 µl of 80% ethanol into the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through and the collection tube.**
10. **Place the RNeasy MinElute spin column into a new 2 ml collection tube. Open the lid, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm).**
11. **Place the RNeasy MinElute spin column into a 1.5 ml collection tube, and pipet 14 µl RNase-free water onto the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the miRNA.**

Purifying total RNA (>200 nt) using the RNeasy Mini Kit

12. **Pipet 350 µl Buffer RW1 into the RNeasy Mini spin column from step 3. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**

    Optional: If on-column DNase treatment using the RNase-free DNase and Buffer RDD is not desired, increase the amount of Buffer RW1 in this step to 700 µl, centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the membrane, and discard the flow-through and collection tube. Then proceed to step 16.

13. **Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix gently by inverting the tube.**

    Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.

14. **Pipet the DNase I incubation mix (80 µl) directly onto the membrane of the RNeasy Mini spin column, and place on the benchtop at room temperature (15–25°C) for 15 min.**

    Note: Make sure to pipet the DNase I incubation mix directly onto the spin column membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

15. **Pipet 350 µl Buffer RW1 into the RNeasy Mini spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through and the collection tube.**

16. **Place the RNeasy Mini spin column into a new 2 ml collection tube. Pipet 500 µl Buffer RPE into the spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**

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

17. **Pipet another 500 µl Buffer RPE into the RNeasy Mini spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and the collection tube.**

18. **Place the RNeasy Mini spin column into a new 2 ml collection tube. Open the lid, and centrifuge at full speed for 1 min.**

19. **Place the RNeasy Mini spin column into a 1.5 ml collection tube. Pipet 30–50 µl RNase-free water directly onto the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute the total RNA.**

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