MQ microRNA Extraction Kit

Cat No. MRE-M-001-50
Size: 50 Preparations

MOLEQULE-ON®

Components	Quantity
miRNA Extractor	60 ml
RPE Solution*	12 ml
RNase Free Water	5 ml
MQ Spin Column TR with 2ml Collection Tube	50
MQ Spin Column MR with 2ml Collection Tube	50

Preparations

*RPE Solution is supplied in a concentrated form, before use, add 4 volumes of 100% ethanol into concentrated RPE solution and mix well.
miRNA Extractor is harmful on contact with skin or if swallowed—please avoid contact with eyes, skin, and clothes. Wash thoroughly after handling and seek professional help if necessary.

Description

MQ microRNA Extraction Kit is used for the isolation of miRNA, siRNA, snRNA (<200 nt) and total RNA. This miRNA Extractor performs well with both small and large quantities of tissue and cells from human, animal, plant or bacterial origin. The kit provides a rapid and efficient method for purifying and enriching miRNA along with other small RNAs, allowing researchers to obtain high-quality miRNA directly from cells or tissues as rapidly and simply as a total RNA prep.

Features

- Optimized for purification of microRNA and other small RNA directly from diverse biological sources.
- Fast and efficient. The entire procedure can be completed in 30 minutes.
- High-purity and high-yield microRNA.
- The purified microRNA can be used subsequently in many applications.

Storage

Shipped at room temperature, store at 2-8°C and protect from light.

Materials and Equipment Required but Not Supplied

- Microcentrifuge capable of at least 12,000 × g
- RNase-free pipettes and pipette tips
- RNase-free microcentrifuge tubes (1.5 ml or 2 ml)
- Vortexer
- RNase-Free Ethanol (96- 100%)
- Chloroform.

Before Starting

- RPE Solution is supplied in a concentrated form, before use, add 4 volumes of 100% ethanol into concentrated RPE solution and mix well.
- Care must be taken when working with RNA. It is important to maintain an RNase-Free environment starting with RNA sample preparation and continuing through purification and analysis. Use RNase free tubes, tips, and gels. Wear gloves at all times.
- Homogenized cell lysates (before adding chloroform) can be stored at -70°C for several months.

Procedure

1. Sample preparation:

Plant Tissue:

Grind ~50-100 mg plant tissue to a fine powder in liquid nitrogen. Transfer the powder to a 1.5 ml RNase- free centrifuge tube. Add 1 ml miRNA Extractor, mix immediately by inverting the tube.

Animal Tissue:

Cut the tissue into small pieces. Add 1 ml miRNA Extractor per every ~25-50 mg tissue. Homogenize for 30 seconds using a homogenizer.

Adherent Cells:

Discard the culture medium carefully. Add 1 ml miRNA Extractor per ~10 cm^2 cultured cells (approximately 10^7 cells). Mix gently by pipetting up and down.

Suspension Cells:

Collect cells by centrifuge, discard the supernatant. Add 1 ml miRNA Extractor for 10^7 cells, mix gently by pipetting. The amount of cells should not be exceed 1×10^7 for fibroblasts or carcinoma cell.

Blood:

Add 0.5 ml fresh anticoagulated whole blood to a 1.5 ml RNase-free centrifuge tube. Centrifuge at 10,000 x g for 2 minutes at room temperature, discard the supernatant (plasma). Add 0.5 ml RNase-free Water and mix by inverting. Centrifuge at 10,000 x g for 2 minutes at room temperature, discard the supernatant. Add 1 ml miRNA Extractor, mix by inverting the tube immediately.

Procedure 1 (Isolation of Total RNA):

1. Incubate samples (with miRNA Extractor) at room temperature for 5-10 minutes to ensure samples are completely lysed.
2. Add 0.2 ml chloroform, vortex for 30 seconds. Centrifuge at 12,000 × g for 10 minutes at 4°C.
   NOTE: After centrifugation, the sample separates into 3 phases. The upper, colorless, aqueous phase contains the RNA.
   NOTE: After centrifugation, a white inter-phase containing DNA and protein may appear. Avoid transferring this layer to reduce DNA contamination.
3. Transfer the supernatant (approximately 540 µl) into a clean 1.5 ml RNase-free centrifuge tube, add 1.5 volumes (usually 810 µl) of 100% ethanol and mix thoroughly by pipetting up and down several times.
   NOTE: Do not centrifuge. A precipitate may form after addition of ethanol, but this will not affect the yield.
4. Transfer the solution to the MQ Spin Column TR, centrifuge at 12,000 × g for 2 minutes, discard the flow-through.
   NOTE: If the volume of lysate exceeds 750 µl, add 750 µl to the column, centrifuge for 30 seconds and discard liquid. Add the remaining lysate to the same column and centrifuge again as above.
5. Add 0.5 ml of RPE Solution to the MQ Spin Column TR, centrifuge at 12,000 × g for 30 seconds, discard the flow-through.
   NOTE: Check the label to ensure the RPE Solution has been diluted with ethanol.
6. Repeat step 5 once.
7. Centrifuge the column at 12,000 × g for 30 seconds.
   NOTE: This step is very important to remove the residual ethanol thoroughly.
8. Place the column to a new 1.5 ml centrifuge tube, add 30-50 µl RNase-free Water, and let it stand for 2 minutes.
9. Centrifuge at 12,000 × g for 30 seconds, save the eluted RNA solution at -80°C.

Procedure 2 (Isolation of microRNA)

1. Incubate samples (with miRNA Extractor) at room temperature for 5-10 minutes to ensure samples are completely lysed.

2. Add 0.2 ml chloroform, vortexing for 30 seconds. Centrifuge at 12,000 × g for 10 minutes at 4°C.
   NOTE: After centrifugation, the sample separates into 3 phases. The upper, colorless, aqueous phase contains the RNA.
   NOTE: After centrifugation, a white inter-phase containing DNA and protein may appear. Avoid transferring this layer to reduce DNA contamination.

3. Transfer the supernatant (approximately 540 µl) into a clean 1.5 ml RNase-free centrifuge tube, add 1/3 volumes (usually 180 µl) of 100% ethanol and mix thoroughly by pipetting up and down several times.
   NOTE: Do not centrifuge. A precipitate may form after addition of ethanol, but this will not affect the yield.

4. Transfer the solution to the MQ Spin Column TR centrifuge at 12,000 × g for 2 minutes, transfer the flow-through (usually 690 µl) to a new 1.5 ml RNase-free centrifuge tube.

5. Add 2/3 volumes of 100% ethanol (e.g. if 690 µl flow- through, add 460 µl ethanol) and mix thoroughly by pipetting up and down several times. Transfer the solution to the MQ Spin Column MR and centrifuge at 12,000 × g for 2 minutes, discard the flow-through.
   NOTE: If the volume of lysate exceeds 750 µl, add 750 µl to the column, centrifuge for 30 seconds and discard liquid. Add the remaining lysate to the same column and centrifuge as above.

6. Add 0.5 ml of RPE Solution to the Spin Column, centrifuge at 12,000 × g for 30 seconds, discard the flow-through.
   NOTE: Check the label to ensure RPE Solution was diluted with ethanol.

7. Repeat step 6 once.

8. Centrifuge the column at 12,000 × g for 30 seconds.
   NOTE: This step is very important to remove the residual ethanol thoroughly.

9. Put the column to a new 1.5 ml centrifuge tube, add 30-50 µl RNase-free Water. Let it stand for 2 minutes. Centrifuge at 12,000 × g for 30 seconds, save the eluted RNA solution at -80°C.