# *EasyPure*® miRNA Kit

Please read the manual carefully before use

**Cat. No.** ER601

**Storage:** LB10 at 2-8℃ away from light for one year; others at room temperature (15℃-25℃) in a dry place for one year

**Description**

This product is suitable for isolating miRNA and total RNA from cells, tissues, fresh blood and exosomes. After samples are lysed with lysis buffer, addition of chloroform to the lysed sample separates the solution to an upper colorless aqueous phase containing RNA, an interphase and a lower pink organic phase. After addition of ethanol to the transferred aqueous phase, total RNA can be specifically immobilized by an RNA Spin Column. By adjusting the volume of ethanol added to the aqueous phase, large RNAs (including 28S rRNA, 18S rRNA and mRNA) are immobilized while small RNAs (≤ 200 nt, such as miRNA, siRNA, shRNA, snRNA, etc.) are in the flow-through which is passed through a miRNA Spin Column where the miRNAs become immobilized after addition of more ethanol. This product possesses advantages of high lysis capacity, high yield and broad applications.

**Kit Contents**

| Component | ER601-01 (50 rxns) |
|---|---|
| Lysis Buffer10 (LB10) | 55 ml |
| Wash Buffer 10 (WB10) | 12 ml |
| RNA Spin Columns with Collection Tubes | 50 |
| miRNA Spin Columns with Collection Tubes | 50 |
| RNase-free Tube (1.5ml) | 50 |
| RNase-free Water | 10 ml |

**Sample Requirements**

| Material | Amount |
|---|---|
| Tissue | 50-100 mg |
| Cell | $1\times10^7$ cells |
| Fresh Blood/Exosome | 50-200 μl |

**Procedures**

Please set refrigerated centrifuge at 2-8℃ in advance, and add 48 ml of absolute ethanol to WB10 prior to use.

Materials needed: chloroform, absolute ethanol.

Sample processing:

1. Homogenization
   a. Adherent cells
      • Discard the culture medium and wash the culture dish once with 1×PBS.
      • Add LB10 to the culture dish (1 ml per 10 $cm^2$). Incubate horizontally for a while to distribute lysis buffer homogeneously on cells and disrupt cells. Detach cells by pipetting (for strongly adherent cells, detach cells with cell spatula).
      • Transfer the lysate containing cells to a microcentrifuge tube. Repeatedly pipette up and down until the lysate contains no visible precipitate.
      • Incubate at room temperature for 5 minutes.
   b. Suspension cells
      • Transfer suspension cells and the culture medium to a microcentrifuge tube. Centrifuge at 8,000×g for 2 minutes at 2-8 ℃, and discard the supernatant.
      • Add LB10 to the tube (1 ml per $1\times10^7$ cells).
      • Repeatedly pipette up and down until no visible precipitates are present in the lysate.
      • Incubate at room temperature for 5 minutes.
   c. Animal and plant tissues
      • After weighing the ultra-low temperature freezing samples, quickly transfer into a precooled mortar with liquid nitrogen. Grind thoroughly to a powder. Add more liquid nitrogen if needed. Incomplete grinding can affect RNA yield and quality.
      • Transfer the tissue powder to a microcentrifuge tube. Add LB10 (1 ml per 50-100 mg tissue). Homogenize tissue samples with a homogenizer or repeatedly pipette up and down to mix well.
      • Incubate at room temperature for 5 minutes.

d. Blood
- Add LB10 to the sample(1 ml per ≤200 µl blood), and mix thoroughly by vortexing.
- Incubate at room temperature for 5 minutes.

e. Exosomes
- Resuspend exosomes with 100 µl PBS, add 1 ml LB10, and vortex to mix well.
- Incubate at room temperature for 5 minutes.

2. Add 0.2 ml of chloroform or 50 µl of 4-Bromoanisole per 1 ml LB10. Vortex the tube vigorously for 30 seconds. Incubate at room temperature for 3 minutes.
3. Centrifuge at 10,000×g for 15 minutes at 2-8°C. The mixture is separated into a lower pink organic phase, an interphase, and an upper colorless aqueous phase which contains RNA. The volume of the aqueous phase is about 1/2-3/5 volume of LB10 reagent used (to avoid DNA contamination due to pipetting the interphase, a portion of aqueous phase can be left in the tube).
4. Transfer the colorless aqueous phase to a new RNase-free tube. miRNA and total RNA can be obtained by two different processing methods.

All following centrifugation steps can be carried out at room temperature.

miRNA purification

(1) Add 1/3 volume of absolute ethanol to the transferred aqueous phase (e.g. for 500 µl transferred aqueous phase, add 167 µl absolute ethanol.) Some precipitates may appear at this moment. Invert the tube to mix well.
(2) Add all the solution and precipitates into the RNA Spin Column, centrifuge at 12,000×g for 30 seconds at room temperature, and collect the flow-through.
(3) Measure the volume of the flow-through accurately and transfer it to a clean 1.5 ml or 2 ml RNase-free centrifuge tube. Add 1.25 volumes of absolute ethanol to the tube (e.g. add 812.5 µl of absolute ethanol to 650 µl of flow-through). Some precipitates may appear at this moment. Invert the tube gently to mix well.
(4) Add all the solution and precipitates into the miRNA Spin Column. Centrifuge at 12,000×g for 30 seconds at room temperature, and discard the flow-through (If the volume of the mixture is larger than the maximum sample volume the column can process, repeat this step until all the mixture has been loaded).
(5) Add 500 µl of WB10 (check to make sure that absolute ethanol has been added prior to use) into the spin column. Centrifuge at 12,000×g for 30 seconds at room temperature. Discard the flow-through.
(6) Repeat step (5) once.
(7) Centrifuge at 12,000 ×g for 2 minutes at room temperature to remove ethanol residue thoroughly.
(8) Place the miRNA spin column into a 1.5 ml RNase-free tube. Add 30-50 µl of RNase-free Water to the center of the spin column matrix and incubate at room temperature for 1 minute.
(9) Centrifuge at 12,000×g for 1 minute to elute miRNA.
(10) Store the isolated miRNA at -80°C.

Total RNA purification

(1) Add 1.25 volumes of absolute ethanol to the transferred aqueous phase (e.g. for 500 µL transferred aqueous phase, add 625 µl absolute ethanol.) Some precipitates may appear at this moment. Invert the tube gently to mix well.
(2) Add all the solution and precipitates into the RNA Spin Column. Centrifuge at 12,000×g for 30 seconds at room temperature, and discard the flow-through (If the volume of the mixture is larger than the maximum sample volume the column can process, repeat this step until all the mixture has been loaded).
(3) Add 500 µl of WB10 (check to make sure that absolute ethanol has been added prior to use). Centrifuge at 12,000×g for 30 seconds at room temperature. Discard the flow-through.
(4) Repeat step (3) once.
(5) Centrifuge at 12,000 ×g for 2 minutes at room temperature to remove ethanol residue thoroughly.
(6) Place the RNA Spin Column into a 1.5 ml RNase-free tube. Add 100 µl of RNase-free Water to the center of the spin column matrix and incubate at room temperature for 1 minute.
(7) Centrifuge at 12,000×g for 2 minute to elute total RNA.
(8) Store the isolated total RNA at -80°C.

**Notes**

- It is important to vortex thoroughly after adding chloroform to ensure good isolation result.
- Ensure that all the organic reagents (including chloroform, absolute ethanol, etc.) and consumables used (such as centrifuge tubes and pipette tips) are RNase-free.
- Isolated miRNA cannot be quantified with a spectrophotometer.

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