miRNA Kit

Catalog No.: abx098094

Size: 50 rxns

Storage: Store the Lysis Buffer in the dark between 2-8 °C for up to 12 months. Store the other kit components dry at room temperature (15-25 °C) for up to 12 months.

Introduction

Abbexa's miRNA Kit is a fast and easy column-based method for the isolation of miRNA and total RNA from cells, tissues, blood, and exosomes. After cell lysis, addition of chloroform separates the lysed sample into an organic phase, interphase, and an aqueous phase containing the RNA. After addition of ethanol to the transferred aqueous phase, total RNA can be immobilized by an RNA Spin Column. By adjusting the volume of ethanol added to the aqueous phase, large RNAs (e.g. 28S rRNA, 18S rRNA and mRNA) can be immobilized while small RNAs (≤ 200 nt, e.g. miRNA, siRNA, shRNA, snRNA) pass into the flow-through. miRNA can be further isolated using a miRNA Spin Column.

Kit components

1. Lysis Buffer: 55 ml
2. Wash Buffer: 12 ml
3. RNase-Free Water: 10 ml
4. RNA Spin Columns with Collection Tubes: 50
5. miRNA Spin Columns with Collection Tubes: 50
6. RNase-Free Tubes: 50

Material Required But Not Provided

1. Absolute ethanol
2. Chloroform or 4-bromoanisole
3. PBS
4. Liquid Nitrogen
5. Pipettes and pipette tips
6. Centrifuge and centrifuge tubes
7. Vortexer
8. Homogenizer

Protocol

A. Reagent Preparation

- **Working Wash Buffer:** Dilute the Wash Buffer 5-fold (1/5) with absolute ethanol before use (i.e. add 48 ml of absolute ethanol to 12 ml of Wash Buffer to prepare 60 ml of Working Wash Buffer).

B. Sample Preparation

- **Adherent cells:** Discard the culture medium and wash the culture dish once with 1X PBS. Add 1 ml of Lysis Buffer for every 10 cm^2 of culture dish, ensuring that the Lysis Buffer is distributed evenly. Allow to stand at room temperature for a few minutes, then detach cells by pipetting (strongly adherent cells may require a cell scraper). Transfer the lysate containing cells to a microcentrifuge tube. Repeatedly pipette up and down until the lysate contains no visible precipitate. Allow to stand at room temperature for 5 minutes.

- **Suspension cells:** Transfer suspension cells and culture medium to a microcentrifuge tube. Centrifuge at 8,000 × g for 2 minutes at 2-8 °C, then discard the supernatant. Add 1 ml of Lysis Buffer per 10^7 cells to the tube. Repeatedly pipette up and down until the lysate contains no visible precipitate. Allow to stand at room temperature for 5 minutes.
- **Animal and plant tissues:** Weigh frozen tissue sample and quickly transfer into a mortar with liquid nitrogen. Grind thoroughly to a powder, using more liquid nitrogen if required. Incomplete grinding can affect RNA yield and quality. Transfer the tissue powder to a new microcentrifuge tube. Add 1 ml of Lysis Buffer for every 50-100 mg of tissue. Homogenize tissue samples with a homogenizer and mix thoroughly by repeatedly pipetting up and down. Allow to stand at room temperature for 5 min.
- **Blood:** Add 1 ml of Lysis Buffer per 50-200 µl of blood. Mix thoroughly by vortexing. Allow to stand at room temperature for 5 minutes.
- **Exosomes:** Resuspend exosomes with 100 µl PBS. Add 1 ml of Lysis Buffer, then mix thoroughly by vortexing. Allow to stand at room temperature for 5 minutes.

C. Assay Procedure

1. Add 0.2 ml of chloroform (or 50 µl of 4-bromoanisole) per 1 ml of Lysis Buffer added to the prepared samples. Vortex for 30 seconds, then allow to stand at room temperature for 5 minutes. It is important to mix thoroughly for good isolation results.
2. Centrifuge at 10,000 × g for 15 minutes at 2-8 °C. The mixture should separate into a lower pink organic phase, interphase, and an upper colorless aqueous phase containing the RNA. The volume of the aqueous phase should be 50-60% of the volume of Lysis Buffer used to prepare the samples.
3. Transfer the upper colorless aqueous phase to a new RNase-free tube. Avoid addition of the interphase or lower phase to prevent DNA contamination. miRNA or total RNA can be obtained from the collected aqueous phase.

D. miRNA Purification

4. Add absolute ethanol to the collected aqueous phase at a ratio of 1:3 (e.g. add 167 µl of absolute ethanol to 500 µl of aqueous phase). Mix by inverting the tube. Precipitates may form at this stage.
5. Transfer the resulting solution and precipitates together to an RNA spin column. Centrifuge at 12,000 × g at room temperature for 30 seconds, then collect the flow-through. If the volume of mixture is larger than the capacity of the spin column, repeat this step.
6. Measure the volume of the collected flow-through, then transfer to a clean RNase-free centrifuge tube. Add absolute ethanol to the collected flow-through at a ratio of 5:4 (e.g. add 812.5 µl of absolute ethanol to 650 µl of flow-through). Mix by inverting the tube.
7. Transfer the resulting solution and precipitates together to a miRNA spin column. Centrifuge at 12,000 × g at room temperature for 30 seconds, then discard the flow-through. If the volume of mixture is larger than the capacity of the spin column, repeat this step.
8. Add 500 µl of Working Wash Buffer (containing ethanol) into the spin column. Centrifuge at 12,000 × g at room temperature for 30 seconds, then discard the flow-through. Repeat this step once more for a total of two times.
9. Centrifuge at 12,000 × g at room temperature for 2 minutes to remove any remaining ethanol residue.
10. Place the spin column into a clean 1.5 ml RNase-free tube. Add 30-50 µl of RNase-free Water into the spin column matrix and allow to stand at room temperature for 1 minute.

11. Centrifuge at 12,000 × g for 2 minutes to elute the miRNA.
12. Store the isolated miRNA at -80 °C.

E. Total RNA Purification

4. Add absolute ethanol to the collected aqueous phase at a ratio of 5:4 (e.g. add 625 µl of absolute ethanol to 500 µl of aqueous phase). Mix by inverting the tube. Precipitates may form at this stage.
5. Transfer the resulting solution and precipitates together to an RNA spin column. Centrifuge at 12,000 × g at room temperature for 30 seconds, then discard the flow-through. If the volume of mixture is larger than the capacity of the spin column, repeat this step.
6. Add 500 µl of Working Wash Buffer (containing ethanol) into the spin column. Centrifuge at 12,000 × g at room temperature for 30 seconds, then discard the flow-through. Repeat this step once more for a total of two times.
7. Centrifuge at 12,000 × g at room temperature for 2 minutes to remove any remaining ethanol residue.
8. Place the spin column into a clean 1.5 ml RNase-free tube. Add 30-50 µl of RNase-free Water into the spin column matrix and allow to stand at room temperature for 1 minute.
9. Centrifuge at 12,000 × g for 2 minutes to elute the total RNA.
10. Store the isolated total RNA at -80 °C.

F. Notes

- Incomplete grinding of tissue samples can affect RNA yield and quality.
- Ensure all reagents (e.g. chloroform, absolute ethanol) and consumables (e.g. pipette tips, centrifuge tubes) used are RNase-free.
- Isolated miRNA cannot be quantified with a spectrophotometer.