AllPure miRNA kit

Cat. No. ABTGNA601-50

Storage: LB10 at 4°C in dark for one year, others at room temperature (15°C-25°C) for one year

Description

AllPure miRNA Kit provides a simple and fast column based method to isolate small RNA (≤200 nt) from cells, tissues, fresh blood and virus. Samples are lysed with lysis buffer. The addition of chloroform to the sample separates the solution into an upper colorless aqueous phase containing RNA, an interphase and a lower organic phase. High molecular RNA (28S rRNA, 18S rRNA, mRNA) is bound to a silica membrane. Small RNA in the flow-through can be bound to a miRNA spin column.

Kit Contents

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

Sample Requirement

Material	Amount
Tissue	50-100 mg
Cell	1×10^7 cells
Fresh Blood	50-200 µl

Procedures

Please adjust refrigerated centrifuge to 4°C in advance, and add 48 ml of 96%-100% ethanol to WB10 prior to use.

Materials needed: chloroform, 96%-100% ethanol

1. Homogenization

a. Adherent cells

· Wash culture dish once with 1×PBS.

· Add 1 ml of LB10 to per 10 cm^2 culture dish, incubate horizontally for a while to allow lysis buffer homogeneously to immerse cells and disrupt cells. Passing the cell lysate several times through a pipette tip (for adherent cells, detach cells with cell spatula).

· Transfer lysate containing cells to a microcentrifuge tube. Repeatly pipette up and down to disperse any visible precipitate.

· Incubate at room temperature for 5 minutes.

b. Suspension cells

· Transfer suspension cells from culture dish to a microcentrifuge tube. Centrifuge at 8,000×g for 2 minutes at 4°C, discard the supernatant.

· Add 1 ml of LB10 to per 10^7 cells.

· Repeatly pipette up and down until no visible precipitates are present in lysate.

· Incubate at room temperature for 5 minutes.

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c. Animal tissue and plant materials

· After weighing, quickly transfer frozen sample into mortar with liquid nitrogen. Grind thoroughly to a powder. Add more liquid nitrogen if needed. Incomplete grind can affect RNA yield and quality.

· Transfer the tissue powder to a microcentrifuge tube. Add 1 ml of LB10 to per 50-100 mg tissue. Homogenize tissue samples with a homogenizer and repeatly pipette up and down.

· Incubate at room temperature for 5 minutes.

d. Blood

· Add 1 ml of LB10 to per ≤200 µl blood, mix thoroughly by vortexing.

· Incubate at room temperature for 5 minutes.

2. Add 0.2 ml of chloroform or 50 µl of 4-Bromoanisole to per ml LB10. Shake the tube vigorously by hand for 30 seconds. Incubate at room temperature for 3 minutes.
3. Centrifuge at 10,000×g for 15 minutes at 4°C. The mixture separates into a lower pink organic phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is about 1/2-3/5 volume of LB10 reagent (to avoid DNA contamination, a portion of aqueous phase can be left in the tube).
4. Transfer the colorless, upper phase containing the RNA to a fresh RNase-free tube. Add 96%-100% of ethanol (equal to 1/3 volume of the transferred solution) to 1 volume of the transferred solution (e.g. add 200 µl of 96%-100% of ethanol to 600 µl transferred solution, some precipitates may form at this moment). Mix gently by inverting tube.
   All following centrifugation steps are carried out at room temperature.
5. Add all the lysate into the RNA spin column, centrifuge at 12,000×g for 30 seconds at room temperature, and collect the flow-through.
6. Measure the volume of flow-through and transfer it to a clean 1.5 ml or 2 ml RNase-free tube. Add 96%-100% ethanol (equal to 1.25 volume of flow-through) to the tube (e.g. add 812.5 µl of 96%-100% ethanol to 650 µl of flow-through, some precipitates may be present at this moment). Mix gently by inverting tube.
7. Add the entire lysate into the miRNA spin column, centrifuge at 12,000×g for 30 seconds at room temperature, discard the flow-through (if the lysate volume is more than the loading volume of miRNA spin column, repeat this step until all the lysate is applied).
8. Add 500 µl of WB10 (check to make sure that ethanol has been added) into the spin column. Centrifuge at 12,000×g for 30 seconds at room temperature. Discard the flow-through.
9. Repeat step 8 once.
10. Centrifuge the column at 12,000 ×g for 2 minutes at room temperature in order to remove ethanol residue and then air-dry the miRNA spin column matrix for several minutes.
11. Place the miRNA 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 incubate at room temperature for 1 minute.
12. Centrifuge at 12,000×g for 1 minute to elute miRNA.
13. Store the isolated miRNA at -80°C.

Notes

· It is important to mix well after adding chloroform.

· Isolated miRNA can not be quantified with spectrophotometer.