AFTSpin Micro Fast RNA Extraction Kit

Cat. No. : RK30124

Product components

Components	Component number	Size 50 RXN	Storage
Buffer RL1	RM30140	30 mL	RT
Buffer PR2	RM30141	35 mL	RT
RNase-free H_2O	RM30142	5 mL	RT
Buffer WB2	RM30144	12 mL	RT
Micro RNase-free adsorption column and collection Tubes	RM30208	50 pcs	RT
Micro gDNA remove column and collection Tubes	RM30209	50 pcs	RT
Poly Carrier	RM30206	200 μL	-20℃
Micro pestle	RM30207	3 pcs	RT
1.5 mL RNase-free centrifuge Tubes	RM30202	50 pcs	RT

Product Description

This kit can be used for rapid extraction of RNA from various trace samples, and does not rely on toxic reagents such as phenol and chloroform. The unique lysis buffer rapidly lyses cells from trace samples and inactivates cell RNA enzymes. The lysate mixture passes through a gDNA Remove Column, where gDNA is removed and RNA is filtered through. After adjusting the binding conditions by ethanol, RNA is selectively bound to the silicon membrane of the adsorption column in the state of high disordered salt. In a series of rapid washing-centrifugation steps, the purified RNA is eluted from the silicon membrane with low salt RNase-free H_2O. The extracted RNA can be directly used in RT-PCR, qPCR and RNA library constructing experiments.

Storage

This kit is guaranteed stable for 12 months when stored properly at room temperature. Low temperature storage is easy to cause sediment formation and affect the experimental results. Transportation at room temperature. Poly Carrier can be stored at 4℃ for one month and -20℃ for a long time.

Precautions

1. This product is for scientific research use only by professionals and is not intended for clinical diagnosis or treatment.
2. Please wear a lab coat and disposable gloves for your safety and health.
3. Unless otherwise specified, all centrifugation steps should be completed at room temperature, using a benchtop centrifuge at a speed greater than or equal to 13,000 rpm (~14,000 x g).
4. Buffer RL1 and Buffer PR2 contain irritating compounds. Please wear latex gloves during operation to avoid contamination of skin, eyes and clothing. If skin, eyes are contaminated, rinse with plenty of water or normal saline.
5. After each use, the reagent bottle should be tightly capped immediately to avoid volatilization, oxidation and pH change caused by long-term exposure to the air.
6. The kit removes the vast majority of DNA contamination in the system, and purified RNA can usually be used for downstream experimental operations without DNase I treatment. If downstream experiments are sensitive to trace DNA, DNase I can be used to further remove DNA contamination.
7. Please wear a lab coat, disposable latex gloves, disposable mask, and use RNase-free consumables to avoid RNase contamination.

Operation Instruction

Preparation before the experiment

1. Prior to the first use, add 48 mL of absolute ethanol (self-prepared by the user) to Buffer WB2 and mix thoroughly. Mark the

reagent bottle to indicate that ethanol has already been added.

2. Check Buffer RL1 for precipitate. If there is precipitate, put the tube in a water bath at 65℃ until the precipitate disappears.

User Protocol (please read the precautions first)

1. Sample processing

Note: If the number of cells is less than 5000 or the amount of tissue is less than 10 μg, add 4 μL Poly Carrier to the lysate before homogenization.

A. Cell

a. **Adherent cell**: Thoroughly suck up the culture liquid. Directly add 350μL **Buffer RL1** and then blow it to the cells repeatedly for direct lysis (Perform extraction steps after this cell direct lysis); For culture containers that are not convenient for direct lysis, cells can be scraped off with cell scrapers, or collect cells into 1.5mL centrifuge tubes after trypsin digestion .

Suspension cell: Collect < $5x10^5$ suspension cells into a 1.5mL centrifuge tube (self-provided) and centrifuge at 13,000 rpm for 10 sec (or 300 g for 5 min) to precipitate the cells. Completely remove and discard the supernatant, otherwise it will cause decreased yield and purity.

b. Flick the bottom of the centrifuge tube to make the cells precipitate loosely, add 350 μl **Buffer RL1** (< $5x10^5$ cells), and vortex until there are no obvious cell masses.

c. Perform the following extraction steps immediately.

B. Animal tissue

a. **Electric homogenization**: After adding 350 μL **Buffer RL1** to < 5 mg tissue, thoroughly homogenate for 20-40 sec electrically.

Grinding pestle & homogenization: Add 100μL **Buffer RL1** into a 1.5 mL centrifuge tube, add < 5mg tissue and grind completely with micro pestle immediately, and supplement the **Buffer RL1** to 350 μL.

Liquid nitrogen grinding & homogenization: After grinding into fine powder in liquid nitrogen, transfer appropriate amount of fine powder (<5 mg) into a 1.5 mL centrifuge tube containing 350 μL of **Buffer RL1**, and vortex for 20 sec to fully lysis. Refractory samples can be homogenized by repeated resuspension with a pipette or griding with a micro pestle.

Note: If there are too many insoluble fragments after homogenization, the homogenized lysate can be centrifuged at 13,000 rpm for 3 min to precipitate fragments or insoluble The supernatant is added to the Micro gDNA remove column (the remove column is placed in the collection tube).

b. Perform the following extraction steps immediately.

2. Extraction

a. Add the lysate mixture or homogenate mixture to the Micro gDNA remove column (the remove column is placed in the collection tube).

b. Centrifuge at 13,000 rpm (~14,000 x g) for 30 sec, collect the filtrate (RNA in filtrate).

c. Use a micropipette to accurately estimate the volume of filtrate (usually 350 μL), add 0.5 times the volume of absolute ethanol (precipitation may occur at this time, but it does not affect the extraction process), mix immediately and do not centrifuge.

d. Add the mixture to the Micro RNase-free Adsorption Column placed in the Collection Tube, centrifuge at 13,000 rpm (14,000 x g) for 30 sec, and discard the filtrate.

e. Place back the Micro RNase-free Adsorption Column into the Collection Tube, add 700 μL of **Buffer PR2** to the Adsorption Column, incubate it for 30 sec at room temperature. Then centrifuge at 13,000 rpm (14,000 x g) for 30 sec, and discard the filtrate.

f. Place back the Micro RNase-free Adsorption Column into the collection tube, add 500 µL of **Buffer WB2** (**confirm already adding 48 mL of absolute ethanol prior to the first use**) to the Micro Adsorption Column, centrifuge at 13,000 rpm (14,000 x g) for 30 sec, and discard the filtrate.

g. Repeat Step f. once.

h. Place back the Micro RNase-free Adsorption Column into the collection tube, centrifuge the tube at 13,000 rpm (~14,000 x g) for 2 min to remove the remaining Bufferd WB2 in the Adsorption Column.

i. Remove the RNase-free Adsorption Column and put it into a 1.5mL RNase-free centrifuge tube. Add 10-20 µL of RNase-free H_2O to the middle of the adsorption column and allow to stand at room temperature for 1 min. Centrifuge at 13,000 rpm (14,000 x g) for 1 min to elute the RNA.

Note: Reducing the elution volume (the volume of elute buffer should not be less than 6 µL) can improve the RNA concentration, but the RNA yield will be decreased. User can determine elution volume according to the need.

j. The extracted RNA can be directly used for downstream experiments or stored at -80℃.