AFTSpin Tissue/Cell Fast RNA Extraction Kit for Animal

Cat. No. : RK30120

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
RNase-free Adsorption Column and Collection Tubes	RM30185	50 pk	RT
gDNA Remove Column and Collection Tubes	RM30186	50 pk	RT
1.5 mL RNase-free Centrifuge Tubes	RM30202	50 pk	RT

*Note: Add 48 mL of absolute ethanol to Buffer WB2 bottle prior to initial use.

Product Description

The kit rapidly extracts RNA from animal tissues and cells using silica gel column purification technology that avoids the use of toxic reagents such as phenol and chloroform. Residual gDNA is removed using the included gDNA removal columns. Extracted RNA can be directly used for RT-PCR, qPCR, RNA library construction, and other experiments.

Storage

Reagents are stable at room temperature. Storage at low temperatures should be avoided due to precipitation of reagents which can negatively affect performance.

Highlights

1. Avoids toxic reagents such as phenol and chloroform as well as ethanol precipitation.
2. Extraction of a single sample can generally be completed within 15 minutes.
3. Use of gDNA Removal Columns eliminates the need for DNase digestion of product RNA.
4. Produces reliable results from animal tissue samples as well as animal cells.

Scope of Application

This product enables rapid extraction of total RNA from animal tissues and cells, facilitates removal of gDNA residues, and generates quality RNA for use in RT-qPCR, RT-PCR, and a variety of other applications.

Operational Instructions

Materials to be prepared by the user

Absolute ethanol, 1.5 mL RNase-free centrifuge tubes

Reagent Preparation

1. Buffer WB2: Add 48 mL of absolute ethanol to Buffer WB2 bottle prior to initial use.
2. Prior to use,check Buffer RL1 for precipitation; any precipitate needs to be dissolved in a 37°C water bath. The solution can be used only after it becomes clear.

Precautions

1. This product is for scientific research use by professionals only and is not intended for clinical diagnosis or treatment.
2. All centrifugation steps should be performed at room temperature using a centrifuge at ≥ 12,000 rpm (~13,400 x g).
3. Buffer RL1 and Buffer PR2 contain irritating compounds. Please wear latex gloves while handling to avoid contact with skin, eyes, and clothes. If skin or eyes are contaminated, rinse with large quantities of water or saline.
4. Reagent bottles should be tightly capped immediately after each use to avoid volatilization, oxidation, and pH changes

caused by prolonged exposure to the air.

5. This kit removes the vast majority of contaminating DNA in the purified RNA sample; the RNA can be used for experimental downstream operations without DNase I treatment. Should downstream experiments be very sensitive to DNA, DNase I may be used to further remove DNA contamination.
6. When using this kit, please wear lab coat, disposable latex gloves, disposable mask, and use RNase-free supplies to avoid RNase contamination.

User Protocol

1. Sample treatment

A. Adherent Cell

Trypsin digestion of culture is not required. After removing the supernatant from the medium, add 500 μL Buffer RL1 directly for cell lysis. For culture containers that are not convenient for direct lysis, cells can be scraped off with cell scrapers or collected via aspiration after trypsin digestion; transfer collected cells to a 1.5 mL centrifuge tube, then add 500 μL Buffer RL1 (for $5x10^6$ cells or less) and vortex until no obvious cell mass is visible.

B. Suspension Cell

Centrifuge cellular suspensions at 13,000 rpm (~14,000 g) for 10 s to precipitate cells. Remove supernatant, then add 500 μL Buffer RL1 (for $5×10^6$ cells or less) and vortex until no obvious cell mass is visible.

C. Animal Tissues

a. Homogenate treatment: To 10-20 mg fresh tissue, add 500 μL Buffer RL1 and homogenize with glass or electric tissue homogenizer until there is no obvious tissue mass.

b. Cryogenic grinding: After processing with liquid nitrogen, transfer 10-20 mg of broken tissue material into 1.5 mL RNase-free centrifuge tube, add 500 μL Buffer RL1, and vortex for 20 s. Resuspend with pipette tips as needed to further mix and remove any remaining clumps of sample.

2. RNA extraction

a. After sample disruption, load the sample onto the gDNA Remove Column with the collection tube in place. Centrifuge at 13,000 rpm (~14,000 g) for 1 min, discard the gDNA Remove Column, and retain the filtrate within the collection tube **(RNA in the filtrate).**

b. Add 0.5 times volume of absolute ethanol to the filtrate. Precipitation may occur at this time but will not affect RNA extraction process. Immediately mix ethanol and filtrate using pipette.

c. Add the mixture from extraction step b to a RNase-free Adsorption Column with a collection tube in place, centrifuge at 13,000 rpm (~14,000 g) for 30 sec, and discard the filtrate.

d. Place the RNase-free Adsorption Column back into the collection tube, add 700 μL of Buffer PR2 to the adsorption column, centrifuge at 13,000 rpm (~14,000 g) for 30 sec, and discard the filtrate.

e. Place the RNase-free adsorption column back into the collection tube, add 500 μL of Buffer WB2 (**Confirm addition of absolute ethanol**) to the adsorption column , centrifuge at 13,000 rpm (~14,000 g) for 30 seconds, and discard the filtrate.

f. Repeat extraction step e once.

g. Place the RNase-free adsorption column back into the collection tube, and centrifuge the empty tube at 13,000 rpm (~14,000 g) for 2 minutes to remove any residual Buffer WB2 left in the adsorption column (the empty tube can be placed in a fume hood for 5 minutes until ethanol is completely evaporated).

h. Transfer the RNase-free adsorption column into a clean 1.5 mL RNase-free centrifuge tube, add 30-100 μL of RNase-free ddH_2O to the center of the adsorption column, and allow to stand at room temperature for 2 minutes. Centrifuge at 13,000 rpm (~14,000 g) for 1 minute to elute the RNA.

i. The extracted RNA can be used directly for downstream experiments or stored at -80°C.