BonaPure Animal Tissue Total RNA Extraction Kit

Cat. No. :RE135

Composition

Components	Amount(50 rxns)
Buffer FRL	30 mL
Buffer FRW	40 mL
Buffer FRW1	12 mL
Proteinase K	500 μL
RNase-Free ddH_2O	15 mL
RNase-Free BonaPure RNA Columns（Includes 2.0 mL collection tube）	50 Sets
RNase-Free BonaPure gDNA Remove Columns（Includes 2.0 mL collection tube）	50 Sets

Storage Condition

Store at room temperature (10~25°C) for 12 months, and proteinase K at -20°C.

Introduction

This kit can rapidly extract total RNA from ≤20 mg animal soft tissue (liver, spleen, kidney, brain, etc.), ≤1×107 cultured cells.

The kit is based on silica gel column purification technology, no need to use DTT and β-mercaptoethanol during the extraction process, and the entire extraction process can be completed within 30 minutes.

The kit combines DNA filtration technology, which can efficiently filter and remove genomic DNA.

The extracted total RNA has high purity and is free from protein and other impurities.

It can be used for RT-PCR, Northern Blot, Poly A purification, RNase protection analysis and in vitro translation, etc. experiment.

Highlights

- ✧ Easy to operate: complete the extraction of total RNA from several samples within 30 minutes;
- ✧ Efficient removal of genomic DNA: unique genomic DNA filter column without DNase treatment;
- ✧ Safe and low toxicity: no toxic reagents such as DTT and β-mercaptoethanol;
- ✧ High RNA purity: The extracted RNA has no residual impurities and is suitable for downstream experiments that require high purity and integrity.

Usage

This product is suitable for the extraction of total RNA from animal soft tissue, cells and other samples.

Imporatant Notes

1. Add 48 mL of absolute ethanol to the rinse solution Buffer FRW1 before the first use;
2. Use RNase-free plastic products and pipette tips to avoid cross-contamination, and change new gloves frequently. Because the skin often carries bacteria, sweat and RNase, etc., which may lead to RNA degradation;
3. RNA will not be degraded by RNase when it is in lysate FRL. However, RNase-free plastic and glassware should be used for further processing after extraction. Glassware can be baked at 150°C for 4 hours, and plasticware can be soaked in 0.5 M NaOH for 10 minutes, then thoroughly washed with water and sterilized;
4. RNase-Free ddH2O should be used to prepare solutions such as 70% ethanol (add water to a clean glass bottle, add DEPC to a final concentration of 0.1% (V/V), mix well and place overnight before autoclaving)

Protocol

I. Extraction of total RNA from animal tissues

1. Add 350 μL Buffer FRL for every 10-20 mg tissue, thoroughly homogenize the tissue with an electric homogenizer, then add 10 μL Proteinase K, mix well and place at room temperature for 5 min;
   Note: Spleen tissue is recommended to use 5 mg, and muscle tissue can be increased to 50-100 mg.
2. Centrifuge at 12,000 rpm (~13,400 × g) for 2~5 min, take the supernatant, and proceed as in step 3;

II. Extraction of total RNA from cultured cells

This product can process 102~107cells at a time. When using for the first time, it is recommended to use 2~5 × 106cells. Adjust the cell volume according to the results. But in any case, the cell volume should not exceed 1 × 107.

1. Add an appropriate amount of Buffer FRL to the cell sample and break up the cells;

(1) Suspension cells: centrifuge the collected cells, flick or vortex to loosen the cell pellet, add an appropriate amount of Buffer FRL (see the table below for dosage) and 10 μL Proteinase K, vortex or use a pipette to break up the cells.

Cell Amount	Lysis Buffer FRL
$<5\times10^6$	350 μL
$\geq5\times10^6$	600

(2) Adherent cells: Lyse directly in the culture vessel (the diameter of the vessel does not exceed 10 cm), or use trypsin treatment and centrifuge to collect the cell pellet.

a. Direct lysis method: Discard the culture solution thoroughly, and add an appropriate amount of Buffer FRL (see the table below for dosage) and 10 μL Proteinase K to the culture bottle or dish.

Petri dish diameter	Lysis Buffer FRL
＜6 cm	350 μL
6~10 cm	600 μL

b. Trypsin treatment method: discard the culture medium thoroughly, wash the cells with PBS, remove the PBS, add PBS containing 0.10%~0.25% trypsin to treat the cells, when the cells are detached from the container wall, add the medium containing serum to lose Live trypsin, transfer the cell solution to an RNase-Free centrifuge tube, centrifuge at 300×g for 5 min, collect the cell pellet, carefully aspirate all the supernatant, add an appropriate amount of Buffer FRL (see the following table for dosage) and 10 μL Proteinase K.

Cell Amount	Lysis Buffer FRL
＜5×10^6	350 μL
≥5×10^6	600 μL

2. Centrifuge at 12,000 rpm (~13,400×g) for 2~5 min, take the supernatant, and proceed as in step 3;

Note: Excessive sample volume and insufficient lysis will lead to viscous lysate and clog the gDNA filter column. If the lysate is very viscous, the amount of lysate can be increased appropriately.

3. Put the RNase-Free FlaPure gDNA Remove Column in a 2 mL collection tube, transfer the cell lysate or tissue supernatant to the gDNA filter column, centrifuge at 12,000 rpm (~13,400×g) for 30 s, and retain the filtrate;

Note: The tissue lysate needs to be centrifuged at high speed to remove impurities, and cell debris will cause blockage of the column. When processing some samples, there may be a lipid layer on the surface of the lysate after centrifugation, so try not to absorb these substances by transferring the supernatant. If the gDNA filter column is clogged, the filtrate can be aspirated and added with an appropriate amount of lysate for homogenization, and then transferred to a new gDNA filter column.

4. Discard the gDNA filter column, add an equal volume of 70% ethanol to the filtrate, and pipette 3 to 5 times;
5. Put the RNase-Free FlaPure RNA Column in a 2 mL collection tube, and transfer the solution and the precipitate to the adsorption column. Centrifuge at 12,000 rpm (~13,400×g) for 30 s, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube;
6. If DNase I digestion is not performed, add 700 μL Buffer FRW to the adsorption column, centrifuge at 12,000 rpm (~13,400×g) for 30 s, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube;
7. (Optional) If the RNA purity is more stringent in subsequent experiments, DNase I (need to be ordered separately) can be used for DNase digestion on the membrane;

(1) Add 350 μL Buffer FRW to the RNase-Free adsorption column, centrifuge at 12,000 rpm (~13,400×g) for 30 s, discard the waste liquid, and put the adsorption column back into the collection tube;

(2) Add DNase I working solution to the center of the adsorption column and let it stand at room temperature for 15 min;

(3) Add 350 μL Buffer FRW to the RNase-Free adsorption column, centrifuge at 12,000 rpm (~13,400×g) for 30 s, discard the waste, and put the adsorption column back into the collection tube

8. Add 500 μL Buffer FRW1 (check whether ethanol is added before use) to the adsorption column, let stand at room temperature for 2 minutes, centrifuge at 12,000 rpm (~13,400×g) for 30 s, discard the waste liquid, and put the adsorption column back into the collection tube middle;
9. Repeat step 8;
10. Centrifuge at 12,000 rpm (~13,400×g) for 2 minutes, discard the waste liquid, and place the adsorption column at room temperature for several minutes to completely dry the residual rinse solution in the adsorption material;

Note: The purpose of this step is to remove the residual rinsing solution in the adsorption column. The residual rinsing solution may affect subsequent experiments such as RT.

11. Transfer the adsorption column to a new RNase-Free centrifuge tube, add 30-100 μL RNase-Free ddH2O dropwise to the middle of the adsorption membrane, let stand at room temperature for 2 minutes, and centrifuge at 12,000 rpm (~13,400×g) for 2 minutes. The RNA solution was obtained, and the eluted RNA solution was stored at -80°C.

Note: The volume of elution buffer should not be less than 30 μL, too small volume will affect the recovery efficiency.

Q&A

Q1：Why is the column clogged?

A1：

(1) Excessive sample. The lysate is viscous, reduce the amount of sample or increase the amount of lysate, too much sample will reduce the yield and purity;

(2) Insufficient centrifugation of the lysate. The tissue lysate needs to be centrifuged at high speed to remove impurities. Impurities will cause blockage of the column. Transfer the supernatant so as not to absorb impurities as much as possible;

(3) The centrifuge temperature is too low. The operation of this product is carried out at room temperature.

Q2：Why do gDNA filter columns clog during RNA extraction?

A2：

(1) Increase the number of centrifuges, time or revolutions;

(2) The filtrate can be aspirated and added with an appropriate amount of lysate for homogenization, and then transferred to a new gDNA filter column.