ARPENspin Total RNA Extraction Kit

Kit Components

Cat#	ARP-TRES50
Components	**50 Tests**
Lysis Buffer	50 mL
DNase Stop Buffer	15 mL (add 20 mL ethanol before use)
Wash Buffer	12 mL (add 48 mL ethanol before use)
RElution Buffer	30mL
Spin columns	50
Handbook	1 copy

Storage and transportation

1. The kit can be stored at room temperature. Lysis Buffer should be stored at 2-8°C and has a validity period of 12 months.

The kit is room temperature transportable.

Introduction

This column-type total RNA extraction kit uses an improved Lysis Buffer to enhance the lysis performance of the reagent. Chloroform is added to separate RNA and impurities.

This kit is designed to enhance the adsorption capacity of the column membrane for RNA. The RNA is bonded to the spin column through the use of ethanol. After several rounds of washing, impurities can be effectively removed, resulting in the obtainment of high-quality total RNA with a high degree of purity. It can rapidly isolate and purify total RNA from a variety of samples like blood, animal tissues, microorganisms, cultured cells, and process multiple samples simultaneously. The pure RNA can be used for various applications including Northern blotting, blotting hybridization, poly(A)+ selection, in vitro translation, RNase protect assay, RT-PCR/Real time RT-PCR analysis, and construction of cDNA library.

Apparatus and materials to be prepared by the user

* Sterile 1.5mL microcentrifuge tubes
* Microcentrifuge capable of 14,000rpm
* Vortex mixer
* Sterile 10µL/100µL/1000µL tips
* Absolute ethanol
* Chloroform

Important note

DNase Stop Buffer: Please add the amount of ethanol indicated on the bottle label and mix it thoroughly.
Wash Buffer: Please add the amount of ethanol specified on the bottle label and mix it thoroughly.

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Procedure:

1. Sample processing

a) **Tissues**:

Take fresh or -80℃ frozen animal and plant tissues and grind them into fine powder in liquid nitrogen. Add 1mL Lysis Buffer into 1.5 or 2.0mL microcentrifuge tube, then add 30-50mg frozen sample fine powder, shake and mix well, and incubate at room temperature for 5 minutes.
Alternative ways:
1). Add 1mL of lysis buffer and 30-50mg of tissue to the grinding tube, put it in the grinder and grind it thoroughly, incubate at room temperature for 5 minutes, and then extract it according to the extraction steps in the instructions.
2). Add 1-2mL of lysis buffer and 30-50mg of tissue to the mortar, quickly grind it manually until there are no solid particles, take 1mL of the mixture and transfer it to a 1.5mL centrifuge tube, incubate at room temperature for 5 minutes, and then extract it according to the extraction steps in the instructions.

b) **Blood:** Take a certain amount of fresh blood sample, add 3 times the volume of Lysis Buffer (0.25mL of blood + 0.75mL of Lysis Buffer is recommended), mix well, and incubate at room temperature for 5 minutes.

c) **Culture cells:**
Add Lysis Buffer directly to the culture plate to lyse the cells, add 1mL Lysis Buffer per 10cm^2 areas, and repeatedly pipette until the solution is clear and transparent. Alternatively, after trypsin treatment, transfer the cell solution to a 1.5mL centrifuge tube of RNase-Free, centrifuge at 300 × g for 5min, collect the cell pellet, carefully remove all supernatant, add 1mL Lysis Buffer to the 1.5mL centrifuge tube, mix well, and incubate at room temperature for 5 minutes.

d) **Cell suspension:**
Discard the supernatant by centrifugation, and the cells were collected. 1 mL Lysis Buffer was added to each 5 × 10^6-1 × 10^7 cells, and mixes well by shaking, and incubate at room temperature for 5 minutes.
Notes:
(1) Do not wash cells before adding Lysis Buffer to avoid degradation of RNA.
(2) Some yeasts and bacteria may require grinding with liquid nitrogen.
2. Optional steps: If the sample contains a large amount of protein, fat, polysaccharides, plant tubers, etc., you can centrifuge at 4℃ 12000 rpm for 5 minutes to remove insoluble matter, and transfer the supernatant to a new Centrifuge tube
3. Add 200 μL of chloroform, cap the tube, shake vigorously for 15 seconds, and incubate at room temperature for 2 minutes.
4. Centrifuge at 12,000 rpm for 10 minutes at 4℃. At this time, the sample is divided into three layers: the lower blue organic phase, the middle layer and the upper colorless aqueous phase. The RNA is mainly in the aqueous phase. Transfer the aqueous phase to the new RNase-Free Centrifuge tube.
5. Add absolute ethanol into the aqueous phase. The volume of absolute ethanol added should be 0.5 times volume of the aqueous phase, and mix by inversion (precipitation may occur at this time). Transfer the mixed solution with the precipitate to the spin column, centrifuge at 12,000 rpm for 30 seconds at 4℃, and discard the liquid in the tube. If the liquid cannot be transferred at one time, it needs to be transferred twice.
6. Add 500 μL of DNase Stop Buffer (anhydrous ethanol has been added) to the spin column, centrifuge at 12,000 rpm for 30 seconds at 4℃, and discard the liquid in the tube.
7. Add 500 μL of Wash Buffer (anhydrous ethanol has been added) to the spin column, centrifuge

at 12,000 rpm for 30 seconds at 4℃, and discard the liquid in the tube.
8. Repeat step 7.
9. Transfer the spin column into the tube, centrifuge at 12,000 rpm for 2 minutes at 4℃.
10. Transfer the spin column into a new 1.5 mL RNase-free tube, and add 50-100μL RElution Buffer into the spin column, incubate at room temperature for 1 minute.
11. Centrifuge at 12,000 rpm for 1 minute at 4℃, discard the spin column, the liquid in the 1.5 ml centrifuge tube contained RNA.
12. The extracted RNA can be directly used in various downstream application experiments. If not used immediately, please store at -80℃.

Troubleshooting

1. We ensure the stability of the kit should be 12 mouths when stored at room temperature, and Lysis Buffer should be stored at 2-8℃.
2. Centrifuge at 12,000rpm－14,000rpm at room temperature exception for note.（if possible at 4℃）.
3. For the first using, please add absolute ethanol to the DNase Stop Buffer and Wash Buffer as the volume marked on bottles label and mix them well.
4. Please wear gloves and clean all tips and Eppendorf tubes with DEPC-ddH2O in order to avoid RNase contaminant. If conditions permit, please operate in ultra-clean cabinet.
5. The total RNA should be stored at 4℃ or in the ice bath in order to be used in the downstream experiment, e.g. RT-PCR; or be stored at -80℃. It is suggest avoiding long time storage because RNA is extremely easy to be degraded even with any methods. It is suggested that to store RNA sample in nitrogen or stock buffer in order to ensure purity of RNA.