ARPENspin Total RNA Extraction Kit

Cat# ARP-TRES50

Kit Components

COMPONENT	QUANTITY
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	30 mL
Spin columns	50
Handbook	1 copy

Storage and Transportation

Store Lysis Buffer at 2–8°C. Store all other kit components at room temperature. The kit has a shelf life of 18 months and may be transported at room temperature.

Introduction

This spin-column kit isolates and purifies total RNA from blood, animal tissues, microorganisms, and cultured cells. The improved Lysis Buffer supports sample lysis, and chloroform separates RNA from impurities.

Ethanol promotes RNA binding to the spin-column membrane. Subsequent washing steps remove impurities to obtain purified total RNA. Multiple samples can be processed simultaneously.

The purified RNA is suitable for Northern blotting, blot hybridization, poly(A)+ selection, in vitro translation, RNase protection assays, RT-PCR, real-time RT-PCR, and cDNA library construction.

Before You Begin

Equipment and materials required but not supplied

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

Prepare the wash reagents

Before first use, add absolute ethanol to DNase Stop Buffer and Wash Buffer in the amounts specified on the bottle labels. Mix thoroughly. The quantities listed for this kit are 20 mL for DNase Stop Buffer and 48 mL for Wash Buffer.

Procedure

1. Sample preparation

Prepare the sample using the applicable method below.

Tissues

Grind fresh or −80°C-frozen animal or plant tissue into a fine powder in liquid nitrogen. Add 1 mL of Lysis Buffer to a 1.5 mL or 2.0 mL microcentrifuge tube, then add 30–50 mg of the frozen tissue powder. Shake to mix thoroughly and incubate at room temperature for 5 minutes.

Alternative tissue preparation methods

Mechanical grinding: Add 1 mL of Lysis Buffer and 30–50 mg of tissue to a grinding tube. Grind thoroughly, then incubate at room temperature for 5 minutes. Continue with the extraction procedure.

Manual grinding: Add 1–2 mL of Lysis Buffer and 30–50 mg of tissue to a mortar. Grind quickly until no solid particles remain. Transfer 1 mL of the mixture to a 1.5 mL centrifuge tube and incubate at room temperature for 5 minutes. Continue with the extraction procedure.

Sample Preparation Continued

Blood

Add three volumes of Lysis Buffer to one volume of fresh blood. The recommended quantities are 0.25 mL of blood and 0.75 mL of Lysis Buffer. Mix thoroughly and incubate at room temperature for 5 minutes.

Cultured cells

For direct lysis, add 1 mL of Lysis Buffer per 10 cm^2 of culture area directly to the culture plate. Pipette repeatedly until the solution is clear.

Alternatively, after trypsin treatment, transfer the cell suspension to a 1.5 mL RNase-free centrifuge tube. Centrifuge at 300 × g for 5 minutes to collect the cells. Carefully remove all supernatant, add 1 mL of Lysis Buffer, and mix thoroughly. Incubate at room temperature for 5 minutes.

Cell suspensions

Centrifuge the cell suspension to collect the cells and discard the supernatant. Add 1 mL of Lysis Buffer for every 5 × 10^6–1 × 10^7 cells. Shake to mix thoroughly and incubate at room temperature for 5 minutes.

Sample preparation notes

Do not wash cells before adding Lysis Buffer, as this may lead to RNA degradation. Some yeasts and bacteria may require grinding in liquid nitrogen.

Extraction Procedure

2. **Optional clarification.** For samples containing large amounts of protein, fat, polysaccharides, plant tuber material, or similar components, centrifuge at 12,000 rpm for 5 minutes at 4°C to remove insoluble material. Transfer the supernatant to a new centrifuge tube.
3. **Add chloroform.** Add 200 µL of chloroform and cap the tube. Shake vigorously for 15 seconds, then incubate at room temperature for 2 minutes.
4. **Separate the phases.** Centrifuge at 12,000 rpm for 10 minutes at 4°C. Three layers will form: a lower blue organic phase, an intermediate layer, and an upper colorless aqueous phase. RNA is mainly present in the aqueous phase. Transfer this phase to a new RNase-free centrifuge tube.

Extraction Procedure Continued

5. **Bind the RNA.** Add absolute ethanol equal to 0.5 times the volume of the aqueous phase. Mix by inversion; a precipitate may form. Transfer the mixture, including any precipitate, to the spin column. Centrifuge at 12,000 rpm for 30 seconds at 4°C and discard the flow-through. If the entire mixture cannot be loaded at once, load it in two portions, centrifuging after each loading.
6. **Wash with DNase Stop Buffer.** Add 500 µL of DNase Stop Buffer prepared with ethanol. Centrifuge at 12,000 rpm for 30 seconds at 4°C and discard the flow-through.
7. **Wash with Wash Buffer.** Add 500 µL of Wash Buffer prepared with ethanol. Centrifuge at 12,000 rpm for 30 seconds at 4°C and discard the flow-through.
8. **Repeat the wash.** Repeat step 7.

9. **Centrifuge the column.** Place the spin column in a tube and centrifuge at 12,000 rpm for 2 minutes at 4°C.
10. **Add RElution Buffer.** Transfer the spin column to a new 1.5 mL RNase-free tube. Add 50–100 µL of RElution Buffer to the column and incubate at room temperature for 1 minute.
11. **Collect the RNA.** Centrifuge at 12,000 rpm for 1 minute at 4°C. Discard the spin column. The liquid collected in the 1.5 mL tube contains the purified RNA.
12. **Use or store the RNA.** The extracted RNA may be used immediately in downstream applications. If it will not be used immediately, store it at −80°C.

Additional Handling Notes

1. **Storage and shelf life.** Store Lysis Buffer at 2–8°C and all other kit components at room temperature. The kit has a shelf life of 18 months.
2. **Centrifugation.** Unless otherwise specified, centrifuge at 12,000–14,000 rpm at room temperature. Where possible, use 4°C. Follow the specific temperature and speed stated in each procedure step.
3. **Reagent preparation.** Before first use, add absolute ethanol to DNase Stop Buffer and Wash Buffer in the amounts specified on their labels. Mix thoroughly.
4. **Prevent RNase contamination.** Wear gloves and clean all pipette tips and microcentrifuge tubes with DEPC-treated double-distilled water (DEPC-ddH_2O). If available, work in an ultra-clean cabinet.
5. **Handle purified RNA carefully.** Keep RNA at 4°C or in an ice bath before use in downstream experiments, such as RT-PCR, or store it at −80°C. Avoid prolonged storage because RNA is readily degraded. Storage in nitrogen or stock buffer is recommended to help maintain RNA purity.

Contact Details

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