Spin Bacterial RNA Kit

Cat.No.: HYC341

Product Description

This kit adopts high-specificity RNA-binding spin columns and an optimized proprietary buffer system to rapidly isolate total RNA from bacteria or cultured animal cells. The entire procedure can be finished within 30–40 minutes. The purified total RNA boasts ultra-high purity free of protein and other contaminants, suitable for RT-PCR, Real-Time RT-PCR, microarray analysis, in vitro translation and other downstream experiments.

Components

Components	50 T
DNase I	1000 U
10×Reaction Buffer	1 mL
Buffer RL	35 mL
Buffer RW1	40 mL
Buffer RW2*	11 mL
RNase-Free Water	10 mL
Spin Columns FL with Collection Tubes	50
Spin Columns RM with Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 ml)	100

Storage

Store DNase I and 10× Reaction Buffer at 2–8 °C. All other components should be kept at ambient temperature (10–30 °C).

Required Reagents

Lysozyme, β-mercaptoethanol, anhydrous ethanol (freshly opened or dedicated for RNA extraction)

Instructions

Preparations

1. Guidelines for RNase contamination prevention:

1) Use RNase-free plastic consumables and pipette tips to avoid cross-contamination.

2) Prepare all solutions with RNase-free water.

3) Wear disposable masks and gloves throughout the experiment, and replace gloves frequently.

2. Add β-mercaptoethanol to Buffer RL to a final concentration of 1% before use (add 10 µL β-mercaptoethanol per 1 mL Buffer RL). Buffer RL supplemented with β-mercaptoethanol can be stored at 4 °C for one month. If precipitates form, heat to dissolve completely prior to use.
3. Add anhydrous ethanol to Buffer RW2 as instructed on the bottle label before first use.
4. All centrifugation steps are performed at ambient temperature unless otherwise specified, and all manipulations should be carried out promptly.

Operating Protocol

1. Harvest bacterial pellets by centrifugation at 12,000 rpm (~13,400 × g) for 2 min at 4 °C, with a maximum loading volume of 1×10^9 cells. Completely discard all supernatant carefully.

   Note: Residual supernatant will interfere with subsequent digestion.
2. Fully resuspend cell pellets in 100 µL lysozyme-containing TE buffer and incubate at ambient temperature. See below for detailed buffer composition and incubation duration.

Bacterial Type	Final Concentration of Lysozyme in TE Buffer	Incubation Time
Gram-negative (G^-) Bacteria	400 µg/mL	3–5 min
Gram-positive (G^+) Bacteria	3 mg/mL	5–10 min

3. Add 350 µL Buffer RL (confirm β-mercaptoethanol has been supplemented before use) and vortex thoroughly (insoluble precipitates may form in this step). Transfer the entire mixture including precipitates to Spin Column FL fitted with a collection tube, then centrifuge at 12,000 rpm for 2 min.

4. Add 250 µL anhydrous ethanol to the clarified flow-through and mix well (precipitates may form). Load the entire mixture with precipitates onto Spin Column RM assembled with a collection tube. Centrifuge at 12,000 rpm for 1 min, discard flow-through and reinstall the spin column into the collection tube.
5. Add 350 µL Buffer RW1 to the spin column, centrifuge at 12,000 rpm for 1 min, discard flow-through and reinsert the column.
6. Preparation of DNase I Working Mixture: Mix 52 µL RNase-free water, 8 µL 10× Reaction Buffer and 20 µL DNase I (1 U/µL) to prepare a total 80 µL reaction mixture.
7. Directly load 80 µL DNase I working mixture onto the spin column membrane, and incubate at 20–30 °C for 15 min.
8. Add 350 µL Buffer RW1 to the column, centrifuge at 12,000 rpm for 1 min, discard flow-through and reinstall the column.
9. Add 500 µL Buffer RW2 (verify anhydrous ethanol supplementation before use) to the column, centrifuge at 12,000 rpm for 1 min and discard flow-through.
10. Repeat Step 9 once.
11. Place the spin column back into the collection tube and centrifuge at 12,000 rpm for 2 min. **Note: This step removes residual ethanol from the column, as ethanol carryover inhibits downstream enzymatic reactions (digestion, PCR, etc.).**
12. Transfer the spin column to a new RNase-free collection tube. Pipette 30–50 µL RNase-free water onto the center of the silica membrane, incubate at ambient temperature for 1 min, then centrifuge at 12,000 rpm for 1 min to collect RNA eluate. Store purified RNA at −70 °C to avoid degradation.

    Notes:

    1) The elution volume of RNase-free water must not be less than 30 µL; insufficient volume will lower recovery yield.

    2) Repeat Step 12 with fresh 30–50 µL RNase-free water to increase total RNA yield.

    3) Reload the collected eluate back onto the column and repeat Step 12 to obtain higher RNA concentration.