Spin Universal RNA Plus Kit

Cat. No.: HYC314

Product Description

This column-based total RNA extraction kit is optimized based on TRIsolv reagent, designed to isolate total RNA from animal tissues, plant materials, diverse microorganisms and cultured cells. First, lysis buffer thoroughly lyses and homogenizes samples. Under proprietary high-salt conditions, RNA binds specifically to silica membrane. This mechanism drastically cuts down protein contamination and effectively eliminates organic solvent residues, yielding RNA with superior purity and quality. For RNA assays that require high tolerance to trace DNA contamination, residual genomic DNA can be digested on-column using RNase-Free DNase I. The highly purified RNA obtained is directly applicable to multiple downstream applications, including RT-PCR, Northern Blot, Dot Blot and in vitro translation.

Components

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DNase I (RNase Free)	2×1000 U
10×Reaction Buffer	1000 µL
TRIsolv Reagent	60 mL
TRIsolv Pal	10 mL
Buffer RW1	40 mL
Buffer RW2*	11 mL
RNase-Free Water	10 mL
Spin Columns RM with Collection Tubes	50
RNase-Free Centrifuge Tubes (1.5 mL)	50

Storage

Store TRIsolv Pal and TRIsolv Reagent at 2–8 °C, protected from light. Keep DNase I (RNase Free) and 10× Reaction Buffer at 2–8 °C. All other reagents are stored at room temperature (10–30 °C).

Required Reagents and Equipment

- Absolute Ethanol
- Enzymatic Lysis Buffer (required for genomic DNA extraction of Gram-positive bacteria)
  Composition of Enzymatic Lysis Buffer: 20 mM Tris, pH 8.0, 2 mM Na_2-EDTA, 1.2% Triton X-100, Lysozyme at a final concentration of 20 mg/mL

Instructions

Preparations

1. Use RNase-free plastic consumables and pipette tips to prevent cross-contamination.
2. Prepare all solutions with RNase-free water.
3. Wear disposable masks and gloves during operation; replace gloves frequently throughout the experiment.
4. Avoid repeated freeze-thaw cycles of starting samples, as this will compromise RNA yield and integrity.
5. If precipitates are observed in TRIsolv Reagent before use, incubate the reagent in a 56 °C water bath until fully dissolved.
6. Add anhydrous ethanol to Buffer RW2 as instructed on the bottle label and mix thoroughly prior to first use.
7. All centrifugation steps are performed at ambient temperature unless otherwise specified; complete all manipulations promptly.
8. Instructions for DNase I (RNase Free): Add 500 µL RNase-free water to the cryovial containing DNase I (RNase Free). Mix by gentle inversion; do not vortex. The reconstituted DNase I stock concentration is 2 U/µL, store at −20 ± 5 °C.

Protocol

1. Sample Processing
   1) Tissue samples: Fully grind 30–50 mg tissue in liquid nitrogen and add 1 mL TRIsolv Reagent; alternatively, add 1 mL TRIsolv Reagent to tissue followed by homogenization. Note: Sample volume shall not exceed 10% of the volume of TRIsolv Reagent.
   2) Adherent cultured cells: Discard culture medium, then add 1 mL TRIsolv Reagent per 10 cm^2 cell monolayer.

3) Cell suspension: Centrifuge to collect cell pellet. Add 1 mL TRIsolv Reagent per 5×10^6 cells.
4) Blood samples: For blood preserved in RNA blood storage tubes, transfer the mixture with a pipette and add 1 mL TRIsolv Reagent per 1 mL sample mixture.

2. After adding TRIsolv Reagent, pipette the mixture up and down several times for thorough mixing and complete lysis. Incubate at room temperature for 5 min to fully dissociate nucleic acid-protein complexes.
3. Add TRIsolv Pal at the corresponding volume based on the amount of TRIsolv Reagent added in Step 1 (200 µL TRIsolv Pal per 1 mL TRIsolv Reagent). Tighten the cap, shake vigorously for 15 sec, then incubate at room temperature for 2 min.
4. Centrifuge at 12,000 rpm (~13,400×g) for 10 min at 4 °C. The mixture separates into three phases: red organic phase, interphase and colorless upper aqueous phase. RNA mainly resides in the upper aqueous phase. Transfer the aqueous phase to a new RNase-free centrifuge tube.
5. Add an equal volume of 70% ethanol to the collected aqueous phase and mix by inversion. **Note: Prepare 70% ethanol with anhydrous ethanol and RNase-free water.**
6. Transfer all liquid from the previous step into Spin Column RM fitted with a collection tube. If the liquid volume exceeds column capacity, load in multiple aliquots. Centrifuge at 12,000 rpm for 20 sec, discard flow-through and reinsert the spin column into the collection tube. Add 350 µL Buffer RW1 to the spin column, centrifuge at 12,000 rpm for 20 sec, discard flow-through and reinsert the column.
7. Add another 350 µL Buffer RW1 to the spin column, centrifuge at 12,000 rpm for 20 sec, discard flow-through and reinsert the column.
8. Directly load 80 µL prepared DNase I working solution onto the spin column, then incubate at 20 ± 5 °C for 15 min.
9. Preparation of DNase I working solution: Mix 52 µL RNase-free water, 8 µL 10× Reaction Buffer and 20 µL DNase I stock (2 U/µL).
10. Add 350 µL Buffer RW1 to the spin column, centrifuge at 12,000 rpm for 1 min, discard flow-through and reinsert the column.
11. Add 500 µL Buffer RW2 to the spin column, centrifuge at 12,000 rpm for 20 sec, discard flow-through and reinsert the column.

Note: Confirm anhydrous ethanol has been added to Buffer RW2 as labeled on the bottle before use.

12. Repeat Step 11 once.
13. Centrifuge at 12,000 rpm for 2 min to remove residual liquid, then discard flow-through.
14. Open the spin column lid and air-dry at room temperature for several minutes.

    Note: This step removes residual ethanol inside the column. Ethanol carryover will inhibit downstream enzymatic reactions (digestion, PCR, etc.).
15. Place the spin column into a new RNase-free centrifuge tube. Pipette 30–50 µL RNase-free water onto the center of the column membrane, incubate at room temperature for 1 min, then centrifuge at 12,000 rpm for 1 min to collect RNA eluate. Store purified RNA at −70 ± 10 °C.

    Notes:

    1) The volume of RNase-free water shall not be less than 30 µL, otherwise RNA recovery will decrease.

    2) Repeat Step 15 with another 30–50 µL fresh RNase-free water to increase total RNA yield.

    3) For higher RNA concentration, reload the collected eluate back onto the spin column and repeat the centrifugation step in Step 15.
16. (Optional) Add RNase Inhibitor to the eluted RNA to a final concentration of 1 U/µL for effective suppression of RNA degradation.

    Note: RNase Inhibitor is not supplied with this kit by default.