Spin Tissue RNA Plus Kit

Cat. No.: HYC325

Product Description

This kit combines high-efficiency guanidinium thiocyanate lysis technology with silica membrane purification for high-yield total RNA extraction from animal cells and tissues. The maximum input material is typically 30 mg tissue or 1×10^7 cells. For RNA assays sensitive to trace genomic DNA, residual DNA can be eliminated via on-column digestion using RNase-Free DNase I. The highly pure RNA obtained is applicable to various downstream experiments including RT-PCR, Northern Blot and Dot Blot.

Components

Components	50T
DNase I (RNase Free)	2×1000 U
10×Reaction Buffer	1000 µL
Buffer RL	35 mL
Buffer RW1	30 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 DNase I (RNase Free) and 10× Reaction Buffer at 2–8 °C. All other components should be kept at room temperature (10–30 °C).

Required Reagents

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

Instructions

Preparations

1. Use RNase-free plastic consumables and pipette tips to prevent nuclease contamination.
2. Prepare all solutions with RNase-free water.
3. Wear disposable masks and gloves during operation, and replace gloves frequently throughout the experiment.
4. Avoid repeated freeze-thaw cycles of starting samples, which will compromise RNA yield and integrity.
5. Add β-mercaptoethanol to Buffer RL before use: add 10 μL β-mercaptoethanol per 1 mL Buffer RL. Buffer RL supplemented with β-mercaptoethanol is stable for one month at ambient temperature.
6. Add anhydrous ethanol to Buffer RW2 as instructed on the bottle label and mix thoroughly prior to first use.
7. If precipitates form in Buffer RL, incubate at 56 °C until fully dissolved, then cool to room temperature.
8. Perform all centrifugation steps at ambient temperature; complete all manipulations promptly.
9. 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

a. Tissue samples: Grind tissue thoroughly in liquid nitrogen. Add 600 μL Buffer RL for 20–30 mg tissue, or 350 μL Buffer RL for tissue less than 20 mg. Sample volume must not exceed one-tenth of Buffer RL volume.

b. Adherent cultured cells: Lyse cells directly in culture flasks, or prepare cell suspension and harvest pellet by centrifugation then discard supernatant. Add 600 μL Buffer RL for a growth area of 6–10 cm^2, or 350 μL Buffer RL for area below 6 cm^2. Pipette up and down repeatedly for full mixing and complete lysis.

c. Cell suspension samples: Centrifuge at 12,000 rpm (~13,400×g) for 1 min and discard supernatant to collect cell pellet. Add 600 µL Buffer RL for 5×10^6–1×10^7 cells, or 350 µL Buffer RL for less than 5×10^6 cells. Pipette repeatedly to fully homogenize and lyse cells.

Notes:

1) Remove culture medium completely, as medium components may inhibit cell lysis and reduce RNA yield.

2) Ensure full resuspension and thorough lysis of cells to avoid low RNA yield.

3) Confirm β-mercaptoethanol has been added to Buffer RL before use.

2. After complete lysis, incubate the sample at ambient temperature for 5 min to fully dissociate nucleic acid-protein complexes.
3. Centrifuge at 12,000 rpm for 2–5 min, retain supernatant for subsequent steps.
4. Add an equal volume (600 µL or 350 µL) of 70% ethanol to the supernatant collected in Step 3 and mix well.

Notes:

1) Prepare 70% ethanol with anhydrous ethanol and RNase-free water.

2) Precipitates may form after ethanol addition, which will not interfere with downstream operations.

5. Load the entire mixture onto Spin Column RM pre-assembled with a collection tube; split into two loads if the volume exceeds column capacity. Centrifuge at 12,000 rpm for 1 min, discard flow-through and reinsert the spin column into the collection tube.

Note: Maximum binding capacity of the spin column is 100 µg; overloading will lower RNA yield and purity.

6. Add 350 µL Buffer RW1 to the spin column, centrifuge at 12,000 rpm for 1 min, discard flow-through and reinsert the column.
7. Load 80 µL DNase I working solution onto the spin column, then incubate at 25 ± 5 °C for 15 min.
8. 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).
9. Add 200 µL Buffer RW1 to the spin column, centrifuge at 12,000 rpm for 1 min, discard flow-through and reinsert the column.

10. Add 500 µL Buffer RW2 to the spin column, centrifuge at 12,000 rpm for 1 min, discard flow-through and reinsert the column.
    Note: Verify anhydrous ethanol has been added to Buffer RW2 as labeled on the bottle before use.
11. Repeat Step 10 once.
12. Centrifuge at 12,000 rpm for 2 min and discard all flow-through.
13. Open the spin column lid and air-dry at ambient temperature for several minutes.
    Note: This step removes residual ethanol inside the column. Ethanol carryover inhibits downstream enzymatic reactions (digestion, PCR, etc.).
14. Transfer 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 ambient 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) **Elution volume shall not be less than 30 µL; insufficient volume reduces RNA recovery.**
    2) **Repeat Step 14 with fresh 30–50 µL RNase-free water to maximize total RNA yield.**
    3) **For higher RNA concentration, reload the collected eluate onto the column and repeat the centrifugation step in Step 14.**
15. (Optional) Supplement eluted RNA with RNase Inhibitor to a final concentration of 1 U/µL to effectively suppress RNA degradation.
    Note: RNase Inhibitor is not supplied with this kit by default.