Spin Blood RNA Kit

Cat. No.: HYC315

Product Description

This kit is designed for total RNA isolation from fresh whole blood anticoagulated with citrate, EDTA, heparin or other anticoagulants. It processes up to 1.5 mL whole blood per run and yields highly pure RNA fragments longer than 200 bp. Multiple samples can be processed simultaneously within one hour. This reagent eliminates ultracentrifugation with CsCl purification as well as LiCl or ethanol precipitation steps. No toxic organic solvents such as phenol or chloroform are included. Purified RNA is efficiently stripped of enzymatic inhibitors and contaminants including heme and heparin, and is directly compatible with routine molecular biology experiments, including RT-PCR, Northern Blot, Dot Blot and in vitro translation.

Components

Components	50T
Buffer RBL (10×)	60 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)	50

Storage

All components can be stably stored in a dry place at room temperature (15-30℃).

Required Reagents

β-mercaptoethanol, 70% ethanol (prepared with RNase-free water), anhydrous ethanol

Instructions

Preparations

1. Measures to prevent RNase contamination:

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

2) Bake glassware at 180 °C for 4 hours prior to use; soak plasticware in 0.5 M NaOH for 10 min, rinse thoroughly with water, then autoclave.

3) Prepare all solutions with RNase-free water.

4) Wear disposable masks and gloves during the experiment, and replace gloves frequently.

2. Avoid repeated freeze-thaw cycles of samples, which will reduce RNA yield and integrity. Samples suspended in Buffer RL can be stored at −70 °C for up to one month.
3. Inspect Buffer RL for crystals or precipitates before use; dissolve solids by incubation in a 56 °C water bath. 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 is stable for one month at ambient temperature.
4. Add anhydrous ethanol to Buffer RW2 as instructed on the bottle label prior to first use.
5. This kit is not suitable for RNA extraction from frozen whole blood samples treated with anticoagulants.
6. Dilute 10× Buffer RBL 10-fold with RNase-free water before use, and store the diluted working solution at 2–8 °C.
7. For downstream assays highly sensitive to genomic DNA, treat purified RNA with RNase-free DNase I.
8. All centrifugation steps are carried out at ambient temperature unless otherwise specified. Complete all manipulations promptly.

Protocol

1. Add 5 volumes of 1× Buffer RBL (dilute 10× Buffer RBL 10-fold with RNase-free water before use) to 0.5–1.5 mL fresh anticoagulated whole blood. Mix gently by vortexing or inverting the tube, then incubate on ice for 10–15 min and mix twice during incubation. **Note: The turbid suspension will turn clear upon complete lysis of red blood cells. Extend incubation time up to 20 min if needed.**

2. Centrifuge at 2,100 rpm (~400×g) for 10 min at 4 °C, then carefully aspirate and discard the supernatant.
3. Resuspend the pellet thoroughly by gentle vortexing with 2 volumes of 1× Buffer RBL.
4. Centrifuge at 2,100 rpm for 10 min at 4 °C, then completely aspirate all supernatant.
   Note: Residual supernatant will interfere with lysis and reduce RNA yield.
5. Add Buffer RL (confirm β-mercaptoethanol has been supplemented before use): add 600 µL Buffer RL for 0.5–1.5 mL blood sample, or 350 µL Buffer RL for blood samples less than 0.5 mL, and mix well.
6. Transfer the mixture to Spin Column FL assembled with a collection tube. Centrifuge at 12,000 rpm (~13,400×g) for 2 min, retain the flow-through and discard the filter column.
7. Add an equal volume (600 µL or 350 µL) of 70% ethanol (prepared with RNase-free water) to the collected flow-through and mix thoroughly.
   Note: Precipitates may form after ethanol addition, which does not interfere with downstream procedures.
8. Load the entire mixture onto Spin Column RM fitted with a collection tube; load in multiple aliquots if the volume exceeds column capacity. Centrifuge at 12,000 rpm for 15 sec, discard flow-through and reinsert the spin column into the collection tube.
9. Add 700 µL Buffer RW1 to the spin column, centrifuge at 12,000 rpm for 15 sec, discard flow-through and reinsert the column.
10. Add 500 µL Buffer RW2 (verify anhydrous ethanol has been added prior to use) to the spin column, centrifuge at 12,000 rpm for 15 sec, discard flow-through and reinsert the column.
11. Repeat Step 10 once.
12. Centrifuge at 12,000 rpm for 2 min to remove all liquid waste. Leave the spin column open at ambient temperature for several minutes to fully air-dry.
    Note: This step eliminates residual ethanol inside the column. Ethanol carryover inhibits downstream enzymatic reactions (digestion, PCR, etc.).
13. Place the dried 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 °C to prevent degradation.
    Notes:

1) **Elution volume shall not be less than 30 µL; insufficient volume will lower RNA recovery rate.**
2) **Repeat Step 13 with another 30–50 µL fresh RNase-free water to increase total RNA yield.**
3) **For higher RNA concentration, reload the collected eluate onto the column and repeat Step 13.**