Total RNA Isolation Kit (Blood/Cell/Bacteria)

www.fairbiotech.com

For research use only

Sample : up to 300 µl of whole blood, 10^7 mammalian cells and 10^9 bacterial cells

Yield : up to 30µg

Introduction

The Total RNA Isolation Kit provides a fast, simple, and cost-effective method for isolation of total RNA from whole blood, mammalian cells and bacterial cells. Detergents and chaotropic salt are used to lyse cells and inactivate RNase. The specialized high-salt buffering system further allows all RNA bases to bind to the the glass fiber matrix of the spin column while contaminants pass through the column. Impurities are efficiently washed away, and pure RNA is eluted with REL Buffer without phenol extraction or alcohol precipitation needs. RNA purified with The Total RNA Isolation Kit is suitable for a variety of routine applications including RT-PCR, cDNA Synthesis, Northern Blotting, Differential display, Primer Extension and mRNA Selection. The entire procedure can be completed within 25-40 minutes.

Kit Contents

Catalog No.	RB0004	RB0100	RB0200	RB0300
RL Buffer	4 ml	110 ml	110 ml×2	110 ml×3
RA Buffer	2 ml	45 ml	85 ml	125 ml
RO Buffer	1 ml	25 ml	45 ml	65 ml
W1 Buffer	2 ml	45 ml	85 ml	125 ml
W2 Buffer (Add Ethanol)	300µl×2 (1.2 ml)×2	15 ml (60 ml)	25 ml (100 ml)	25 ml×2 (100ml)×2
REL Buffer	1 ml	10 ml	20 ml	30 ml
RL Columns	4 pcs	100 pcs	200 pcs	300 pcs
Collection Tubes	4 pcs	100 pcs	200 pcs	300 pcs

Quality Control

In accordance with FairBiotech's ISO-certified Total Quality Management System, the quality of the Total RNA Isolation Kit is tested on a lot-to-lot basis to ensure consistent product quality.

Additional requirements

∗Ethanol (96~100%) ∗1.5 ml microcentrifuge tubes ∗14.3 M ß-mercaptoethanol ∗RNase-free pipet tips

∗ For Optional Step (DNA Residue Degradation): Add 2 µl DNAse I (2KU/µl) and 10 µl reaction buffer {300 mM Tris-HCl (pH 7.5), 60 mM $MnCl_2$, 300 µg/ml BSA } to the 50µl final product. Let stand for 10 minutes at room temperature (at 25ºC).

∗ For Gram-positive bacteria sample: lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% TritonX-100; pH 8.0, prepare the lysozyme buffer immediately prior to use)

∗ For Fungus sample: lyticase or zymolase, sorbitol buffer (1.2 M sorbitol;10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5; 35 mM mercaptoethanol)

NOTE

★ Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination.

★ Add ethanol (96–100%) to Buffer W2, **shaking before use** (see bottle label for volume).

★ Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.

★ Buffers RA and W1 contain irritants. Wear gloves when handling these buffers.

Total RNA Isolation Kit Protocol

Step 1 Sample Cells Harvesting

Fresh Blood

- Collect blood in EDTA-Na2 treated collection tubes (or other anticoagulant mixtures).
- Transfer up to **300 µl of blood** to a sterile1.5 ml microcentrifuge tube.
- Add **900 µl of RL Buffer** and mix by inversion.
- Incubate the tube on ice for 10 minutes (invert twice during incubation).
- Centrifuge for 5 minutes at 4,000 x g at 4ºC. **Remove the supernatant completely** and resuspend the cells in **100 µl of RL Buffer** by pipetting the pellet up and down.

Cultured Mammalian Cells

- Transfer cultured mammalian cells (up to 10^7) to a sterile 1.5 ml microcentrifuge tube.
- Centrifuge at 6,000 x g for 1 minute. **Remove the supernatant completely** and resuspend the cells in **100 µl of RL Buffer** by pipetting the pellet up and down.

Gram-Negative Bacterial Cells

- Transfer cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
- Centrifuge at 12,000 x g for 1 minute. **Remove the supernatant completely** and resuspend the cells in **200 µl of RO Buffer** by pipetting the pellet up and down. Incubate at room temperature for 5 minutes.

Gram-Postive Bacterial Cells

- Transfer cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
- Centrifuge at 12,000 x g for 1 minute. **Remove the supernatant completely** and resuspend the cells in **200 µl of lysozyme Buffer** by pipetting the pellet up and down. Incubate at room temperature for 10 minutes.

Fungus Cells

- Transfer fungus cells (up to 10^8) to a sterile 1.5 ml microcentrifuge tube.
- Centrifuge at 6,000 x g for 5 minute. **Remove the supernatant completely** and resuspend the cells in **600 µl of sorbitol Buffer** by pipetting the pellet up and down.
- Add **200 U of lyticase or zymolase**. Incubate at 30ºC for 30 minutes.
- Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast. **Remove the supernatant completely** and resuspend the cells in **200 µl of RO Buffer** by pipetting the pellet. Incubate at room temperature for 5 minutes.

Step 2 Lysis

Fresh Blood/Mammalian Cells

- Add **400 µl of RA Buffer** and 4 µl of ß-mercaptoethanol to the resuspended cells from Step 1 and shake vigorously. Incubate at room temperature for 5 minutes.
- Centrifuge at 16,000 x g for 10 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Bacterial Cells/Fungus Cells

- Add **300 µl of RA Buffer** and 3 µl of ß-mercaptoethanol to the sample lysate from Step 1 and mix by vortex. Incubate at room temperature for 5 minutes.
- Centrifuge at 16,000 x g for 10 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Step 3 Binding

- Add 500 µl of 70% ethanol prepared in ddH_2O (RNase-free and DNase-free) to the sample lysate from Step 2 and shake vigorously (break up any precipitate by pipetting).
- Place a **RL Column** in a **Collection Tube**. Apply 600µl of the mixture to the **RL Column**.
- Centrifuge at 14,000 x g for 1 minute. Discard the flow-through and place the **RL Column** in the same **Collection tube.** Transfer the remaining mixture to the same **RL Column**.
- Centrifuge at 14,000 x g for1 minute. Discard the flow-through and place the **RL Column** in the same **Collection tube.**

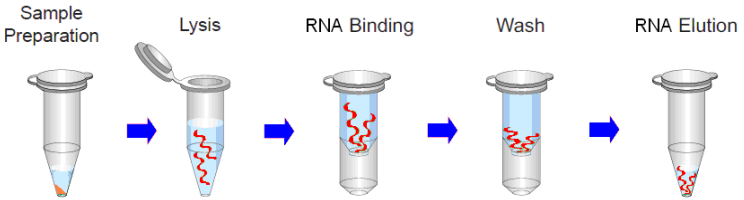
Step 4 Wash

- Add 400 µl of **W1 Buffer** into the **RL Column**. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the **RL Column** back into the same **Collection tube**.
- Add 600 µl of **W2 Buffer** (Ethanol added) into the **RL Column**. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the **RL Column** back into the same **Collection tube.**
- Centrifuge at 14,000 x g again for 2 minutes to remove residual **W2 Buffer**.

Step 5 Elution

- To elute RNA, place the **RL Column** in a clean 1.5 ml microcentrifuge tube.
- Add 50 µl **REL Buffer** to the center of each **RL Column**, let stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.

optional DNase treatments can be followed to remove unwanted DNA residue



Troubleshooting

Problem	Cause	Solution
Degraded RNA / low integrity	RNases contaminantion	Clean everything, use barrier tips, wear gloves and a lab coat, and use RNase-free enzymes, EX: RNase inhibitor.
Low yields of RNA	Incomplete lysis and homogenization	Use the appropriate method for the lysate preparation based on the amount of the starting materials immersed in the Buffer RA to achieve the optimal lysis.
	Incorrect elution conditions	Add 100 µl of the REL Buffer to the center of each RL Column, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA	Repeat the wash step: Centrifuge at 14,000 x g again for 2 minutes to remove the residual W2 Buffer.