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Total RNA Isolation Kit (Tissue)

For research use only

Sample: up to 30 mg of tissue, up to 25 mg of paraffin-embedded tissue

Yield : up to 30µg

Introduction

The Total RNA Isolation Kit provides a fast, simple, and cost-effective method for isolation of total RNA from tissue sample. Detergents and chaotropic salt are used to lyse cells and inactivate RNase. The specialized high-salt buffering system allows RNA species bases to bind to the glass fiber matrix of the spin column while contaminants pass through the column. Impurities are efficiently washed away, and the pure RNA is eluted with REL Buffer without phenol extraction or alcohol precipitation. 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.	RT0004	RT0100	RT0200	RT0300
RR Buffer	2 ml	45 ml	85 ml	125 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
Micropestle	4 pcs	100 pcs	200 pcs	300 pcs
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%) * RNase-free pipet tips and 1.5 ml microcentrifuge tubes * 14.3 M β-mercaptoethanol

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 MnCl₂, 300 µg/ml BSA } to the 50µl final product. Let stand for 10 minutes at room temperature (at 37°C).

NOTE

- ★ Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination.
- ★ Add ethanol (96-100%) to Buffer W2, **shake 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 Preparation

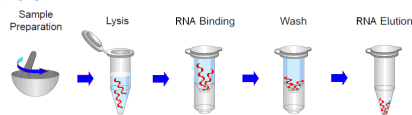
Fresh or Frozen Tissue

- ◆ Cut off up to 30 mg of fresh or frozen animal tissue and grind the sample using one of the micropestles provided in a microcentrifuge tube OR under liquid nitrogen to a fine powder using a mortar and pestle. (If using frozen animal tissue, the sample **MUST** have been flash frozen in liquid nitrogen and immediately stored at -70°C until use, to avoid RNA Degradation).

Paraffin-Embedded Tissue

Additional requirements: xylene, absolute ethanol

- ◆ Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube.
- ◆ Add 1 ml of xylene to the tube. Vortex vigorously and incubate at room temperature for approximately 10 minutes. Vortex occasionally during incubation.
- ◆ Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
- ◆ Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting.
- ◆ Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.



- ◆ Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting.
- ◆ Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
- ◆ Open the tube and Incubate at 37°C for 15 minutes to evaporate any ethanol residue.
- ◆ Proceed with the Lysis Step.

Step 2 Lysis

- ◆ Add **400 µl of RR Buffer** and 4 µl of β-mercaptoethanol to the sample and grind the sample until it is completely dissolved. Transfer the dissolved sample to a RNase-free 1.5 ml microcentrifuge tube. then incubate at 80°C for 20 minutes.
 - ◆ Centrifuge at 16,000 x g for 3 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.
- #Pre-heat the Elution Buffer to 80°C for Step 5 Elution.

Step 3 Binding

- ◆ Add 400 µl of 70% ethanol prepared in ddH₂O (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 for 1 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 of **Pre-Heated 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 contamination	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	Don't use more samples than the suggested limit.
	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.