Total RNA Isolation Kit (Tissue)

Cat No. PDC08-0100
Size: 100 Reactions

Sample: up to 30 mg of tissue, up to 25 mg of paraffin-embedded tissue
Format: Spin column
Operation time: 25-40 minutes
Elution volume: 50 µl
Yield: up to 30 µg

Description

The **Total RNA Isolation Kit (Tissue)** 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

Contents	PDC08-0100	PDC08-0100S
Buffer RR	45 ml	2 ml
Buffer W1	45 ml	2 ml
Buffer W2 (Add ethanol)	15 ml (60 ml)	300 µl x2 (1.5 ml x2)
Buffer REL	10 ml	1 ml
Column RT	100 pcs	4 pcs
Collection Tubes	100 pcs	4 pcs

Feature

- Delivering high-quality total RNA with the fast procedure
- Ready-to-use RNA for high performance in any downstream application
- Consistent RNA yield from the starting material with a small amount

Quality Control

The quality of the Total RNA Isolation Kit (Tissue) is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Ethanol (96~100%)
- 14.3 M ß-mercaptoethanol
- RNase-free pipet tips and 1.5 ml microcentrifuge tubes
- Liquid nitrogen
- For Optional Step (DNA Residue Degradation): Add 2 µl of DNase I (2 KU/ml) mixed a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM $MnCl_2$, 50 µg/ml BSA at 25°C} to the final elution sample. Let it stand for 10 minutes at room temperature
- For Paraffin-Embeded Tissue: xylene, absolute ethanol

Total RNA Isolation Kit (Tissue) Protocol

Step 1 Sample Preparation

Fresh or Frozen Tissue

1. Cut off up to 30 mg of fresh or frozen animal tissue and grind the sample 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).
2. Proceed with the Step2 Lysis.

Paraffin-embedded tissue

1. Slice small sections (up to 25 mg) from blocks of paraffin-embedded tissue and transfer to a 1.5 ml microcentrifuge tube.
2. Add 1 ml of xylene to the tube.
3. Vortex vigorously and incubate at room temperature for approximately 10 minutes.
4. Vortex occasionally during incubation.
5. Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
6. Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting.
7. Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
8. Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting.
9. Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
10. Open the tube and Incubate at 37°C for 15 minutes to evaporate any ethanol residue.
11. Proceed with the Step2 Lysis.

Step 2 Lysis

1. Add 400 µl of Buffer RR and 4 µl of ß-mercaptoethanol to the sample in the mortar and grind the sample until it is completely dissolved.
2. Transfer the dissolved sample to a RNase-free 1.5 ml microcentrifuge tube.
3. Incubate at 80°C for 20 minutes. (invert the tube every 10 minutes)
4. Centrifuge at 14-16,000 x g for 10 minutes.
5. Transfer carefully the clear supernatant to a new 1.5 ml microcentrifuge tube.

Step 3 RNA Binding

1. Add 400 µl of 70% ethanol prepared with ddH_2O (RNase-free and DNase-free) to the sample lysate from Step 2 and shake vigorously (break up any precipitate by pipetting).
2. Place a Column RT in a 2 ml Collection Tube.
3. Transfer the sample mixture to the Column RT.
4. Centrifuge at 14-16,000 x g for 1 minute.
5. Discard the flow-through and and place the Column RT into the same Collection tube.
6. Transfer the remaining mixture to the same Column RT.
7. Centrifuge at 14-16,000 x g for 1 minute.
8. Discard the flow-through and place the Column RT into the same Collection tube.

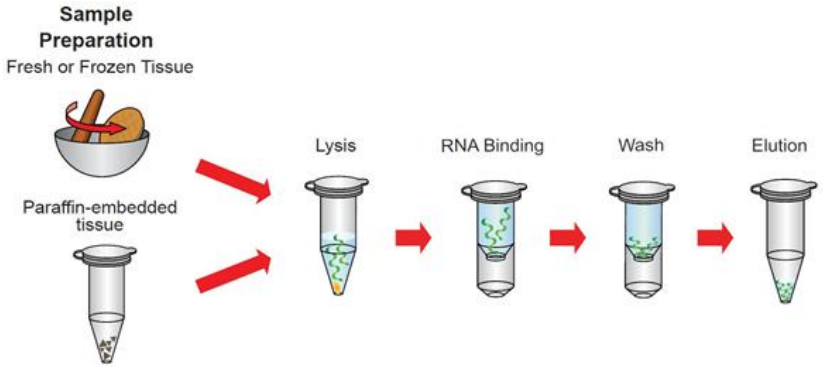
Step 4 Wash

1. Add 400 µl of Buffer W1 into the Column RT.
2. Centrifuge at 14-16,000 x g for 30 seconds.
3. Discard the flow-through and place the Column RT back into the same Collection Tube.
4. Add 600 µl of Buffer W2 (ethanol added) into the Column RT.
5. Centrifuge at 14-16,000 x g for 30 seconds.
6. Discard the flow-through and place the Column RT back into the same Collection Tube.
7. Centrifuge at 14-16,000 x g again for 3 minutes to dry the column matrix.

Step 5 Elution

1. To elute RNA, place the Column RP in a new RNase-free 1.5 ml microcentrifuge tube.
2. Add 50 µl of Buffer REL to the center of each Column RP, let it stand for 2 minutes, and centrifuge at 14-16,000 x g for 2 minutes.

#Optional DNase treatments can be followed to remove the unwanted DNA residue



Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when purifying total RNA with the kit.

Problem	Cause	Solution
Degraded RNA / low integrity	RNases contaminant	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 RP to achieve the optimal lysis.
	Incorrect elution conditions	Add 50 µl of the Buffer RE to the center of each Column RP, 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 Buffer W2.

Caution

- Buffers RR and W1 contain irritants. Wear gloves when handling these buffers.
- Add 60 ml of the ethanol(96~100%) to the Buffer W2 before use.
- Check Buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- During the operation, always wear the latex or vinyl gloves while handling reagents and RNA samples to prevent the RNase contamination.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.