Total RNA Isolation Kit (Tissue)

Cat. No.: SN021-0100 Size: 100 Reactions
Cat. No.: SN021-0004 Size: 4 Reactions
Sample: Up to 30 mg of fresh 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 to isolated 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 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 Buffer RE 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.

Features

- Delivers 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.

Applications

- RT-PCR of RNA.
- Northern blotting.
- Real-time RT-PCR.

Kit Contents

Contents	SN021-0100	SN021-0004
Buffer RR	45 ml X 1 bottle	2 ml X 1 vial
Buffer W1	45 ml X 1 bottle	2 ml X 1 vial
Buffer W2 (add Ethanol)	15 ml X 1 bottle (60 ml X 1 bottle)	300 µl X 2 vials (1.2 ml X 2 vials)
Buffer RE	10 ml X 1 bottle	1 ml X 1 vial
RT Column	50 pieces X 2 bags	4 pieces X 1 bag
Collection Tubes	50 pieces X 2 bags	

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

- Liquid nitrogen
- Mortar and pestle
- 14.3 M ß-mercaptoethanol
- Ethanol (96-100%)
- Water bath/ Dry bath
- Isopropanol
- RNase-free pipet tips and 1.5 ml microcentrifuge tubes
- For Paraffin-Embedded Tissue: xylene, absolute ethanol

Buffer Preparation

For Optional Step (DNA Residue Degradation): Add 2 µl of DNase I (2 KU/ml) mixed in 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.

Total RNA Isolation Kit (Tissue) Protocol

Step 1 Sample Preparation

Fresh or Frozen Tissue

1. Cut off fresh or frozen animal tissue (up to 30 mg) 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 Step 2 Lysis.

Step 2 Lysis

1. Add 1 ml of Buffer RP and 10 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 an RNase-free 1.5 ml microcentrifuge tube.
3. Incubate at 70°C for 30 minutes. (invert the tube every 10 minutes)
4. Centrifuge at 2-8°C at 14-16,000 x g for 10 minutes.
5. Transfer carefully the clear supernatant to a new 1.5 ml microcentrifuge tube.

Paraffin-Embedded Tissue

Additional requirements: xylene, absolute ethanol

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.
6. Remove the supernatant.
7. Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting.
8. Centrifuge at 14-16,000 x g for 3 minutes.
9. Remove the supernatant.
10. Add 1 ml of absolute ethanol to wash the sample pellet again and mix by inverting.
11. Centrifuge at 14-16,000 x g for 3 minutes.
12. Remove the supernatant.
13. Open the tube and Incubate at 37°C for 15 minutes to evaporate any ethanol residue.
14. Proceed with the Step 2 Lysis.

Step 2 Lysis

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

Step 3 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).

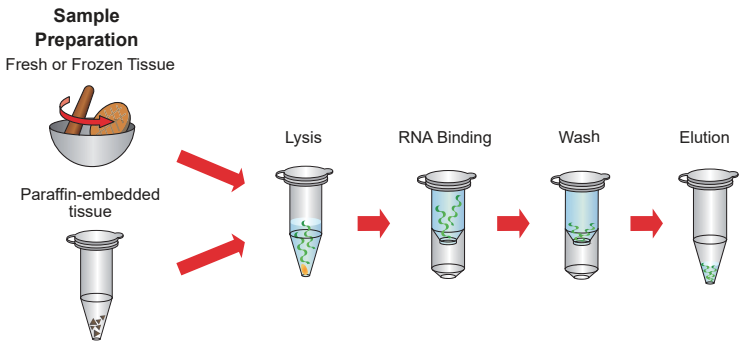
2. Place a RT Column in a Collection Tube.
3. Apply 600 µl of the mixture to the RT Column.
4. Centrifuge at 14,000 x g for 1 minute.
5. Discard the flow-through and place the RT Column into the same Collection tube.
6. Transfer the remaining mixture to the same RT Column.
7. Centrifuge at 14,000 x g for1 minute.
8. Discard the flow-through and place the RT Column into the same Collection tube.

Step 4 Wash

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

Step 5 Elution

1. To elute RNA, place the RT Column in a clean 1.5 ml microcentrifuge tube.
2. Add 50 µl of Buffer RE to the center of each RT Column, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.
3. 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 you did RNA isolation 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, e.g: RiboIN RNase Inhibitor.
Low yields of RNA	Incompletely lysis and homogenization	Use the appropriate method for the lysate preparation based on the amount of the starting materials immersed in the Buffer RR to achieve the optimal lysis.
	Incorrect elution conditions	Add 50 µl of the Buffer RE to the center of each RT Column, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.
Inhibition of do wnstream 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.

Related Ordering Information

Cat. No.	Description	Size
SA001-0500	AGAROSE Tablet, 0.5g	100 Tablets
SL001-1000	Novel Juice Supplied in 6X Loading Buffer	1 ml
SD010-R600	1 Kb DNA Ladder RTU	600 µl
ST040-4000	100 mM dNTP Set	4 x 1 ml
SM701-0050	Oligo(dT)$_{20}$ primer	50 µl
SM305-0050	GScript First-Strand Synthesis Kit	50 Reactions
SM306-0050	GScript One-Step RT-PCR Kit	50 Reactions
SR001-2500	RiboIN RNase Inhibitor	2500 U

Caution

- During the operation, always wear the latex or vinyl gloves while handling reagents and samples to prevent the RNase contamination.
- Check Buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- The Buffers RR and W1 contain irritants, so please wear gloves when handling these buffers.
- When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
- When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to the each vial of the Buffer W2, and shake before use.
- All products are for research use only