Cyrusbioscience

GeneKlean® Total RNA Isolation Kit-Tissue

Cat. Number: TRNAT

Storage: Store at Room Temperature.

Sample: Up to 30mg of tissue or 25mg of paraffin-embedded tissue

Yield: Up to 30ug Total RNA

Kit Contents

Total RNA Isolation Kit-Plant	100 Reactions
GeneKlean® Total RNA Column with 2 mL Collection Tube	100pcs
Lysis Buffer	45mL
Wash Buffer 1	45mL
Wash Buffer 2 (add Ethanol)	15mL (60mL)
Elution Buffer	10mL
Micropestle	100pcs
This Datasheet	1 copy

Note

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination.
- Add ethanol (>99.5%) to Wash Buffer 2, shake before use (see bottle label for volume).
- Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.
- Buffers contain irritants. Wear gloves when handling these buffers.

Additional requirements

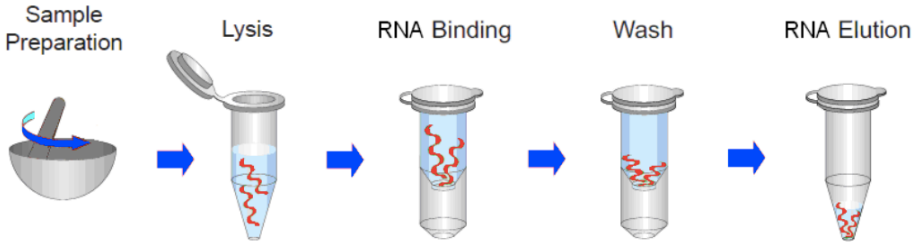
- Ethanol (>99.5%)
- RNase-free pipet tips and 1.5 ml microcentrifuge tubes
- b-mercaptoethanol

Description

GeneKlean® Total RNA Isolation Kit-Tissue provides a fast, simple, and cost-effective method for isolation of total RNA from tissue samples. 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 the glass fiber matrix of the spin column. GeneKlean® Total RNA Isolation Kit-Tissue 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.

Procedure for Preparation of Tissue Total RNA Isolation



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 microtube 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**

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. Vortex vigorously and incubate at room temperature for approximately 10 minutes. Vortex occasionally during incubation.
3. Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
4. Add 1 ml of absolute ethanol to wash the sample pellet and mix by inverting.
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 again and mix by inverting.
7. Centrifuge at 14-16,000 x g for 3 minutes. Remove the supernatant.
8. Open the tube and Incubate at 37°C for 15 minutes to evaporate any ethanol residue.
9. Proceed with the Lysis Step.

Step 1. Lysis

1. Add 400uL of Lysis Buffer and 4uL of b-Mercaptoethanol to the sample and grind the sample until it is dissolved completely. Transfer the dissolved sample to RNase-free 1.5mL microtube. Incubate at 80°C for 20 mins.
2. Centrifuge at 16,000 x g for 3 mins. Transfer the supernatant to a new 1.5mL microtube. ***Pre-heating the Elution Buffer to 80°C for step 4. Elution.**

Step 2. RNA Binding

1. Add 400 µl of 70% ethanol prepared in ddH2O (RNase-free and DNase-free) to the sample lysate from Step 1. Lysis and shake vigorously (break up any precipitate by pipetting).
2. Place a GeneKlean® Total RNA Column in a 2mL Collection Tube. Apply 600uL of the mixture to GeneKlean® Total RNA Column.
3. Centrifuge at 15,000 x g for 1 minute. Discard the flow-through and place the GeneKlean® Total RNA Column in the same Collection tube. Transfer the remaining mixture to the same column.
4. Centrifuge at 15,000x g for 1 min. Discard the flow-through and place the same Total RNA Column back to the same 2mL Collection Tube.

Step 3. Washing

1. Add 400uL of Wash Buffer 1 to the GeneKlean® Total RNA Column. Centrifuge at 15,000x g for 30 secs. Discard the flow-through and place the same Total RNA Column back to the same 2mL Collection Tube.
2. Add 600uL of Wash Buffer 2(ethanol added) into GeneKlean® Total RNA Column. Centrifuge at 15,000x g for 30 secs. Discard the flow-through and place the GeneKlean® Total RNA Column back to the same 2mL Collection Tube.
3. Centrifuge again for 2 mins at 15,000x g to dry the column matrix.

Step 4. Elution

1. Place GeneKlean® Total RNA Column in a clean 1.5mL microtube.
2. Add 50uL of Pre-heated Elution Buffer to the center of GeneKlean® Total RNA Column, stand for 2 mins. Centrifuge 2 mins at 15,000x g.
   *Optional DNase treatments can be followed to remove unwanted DNA residue.

Troubleshooting

Problems	Possible Causes	Suggestions
Degraded RNA/Low integrity	RNase 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 uL of the Elution Buffer to the center of each Column, let it stand for 2 minutes, and centrifuge at 15,000 x g for 2 minutes.
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA	Repeat the wash step: Centrifuge at 15,000 x g again for 2 minutes to remove the residual of Wash Buffer 2.