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Total RNA Extraction 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 BioDiamond Total RNA Extraction 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 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 BioDiamond Total RNA Extraction 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.	DMRTS100	DMRTS300
RR Buffer	45 ml	125 ml
W1 Buffer	45 ml	125 ml
W2 Buffer (Add Ethanol)	15 ml (60 ml)	50 ml (200 ml)
REL Buffer	10 ml	30 ml
RL Columns	100 pcs	300 pcs
Collection Tubes	100 pcs	300 pcs

Quality Control

In accordance with FairBiotech's ISO-certified Total Quality Management System, the quality of the BioDiamond 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 ß-mercaptoethanol

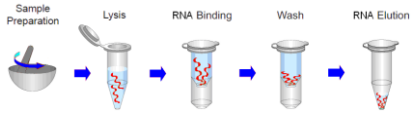
For Optional Step (DNA Residue Degradation):

Add 2 μl DNAse I (2KU/μl) and 10 μl reaction buffer {300 mM Tris-HCl (pH 7.5), 60 mM $MnCl_2$, 300 μg/ml BSA } to the 50μl final product. Let stand for 10 minutes at room temperature (at 37ºC).

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.

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 -70ºC 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 37ºC 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 ß-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 80ºC 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 80ºC 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 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 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 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	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.