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Total RNA Extraction Kit (Plant)

For research use only

Sample: 100 mg of fresh plant tissue or 25 mg of dry plant 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 plant samples. Detergents and chaotropic salt are used to lyse cells and inactivate RNase. The specialized high-salt buffering system allows RNA species longer than 100 bases to bind to the the glass fiber matrix of the spin column . 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 60 minutes.

Kit Contents

Catalog No.	DMRPL100	DMRPL300
RP Buffer	110 ml	320 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
RP 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 Extraction Kit is tested on a lot-to-lot basis to ensure consistent product quality.

Additional requirements

＊Ethanol (96~100%)＊Isopropanol＊RNase-free pipet tips and 1.5 ml microcentrifuge tubes＊ß-mercaptoethanolc

#For Optional Step (DNA Residue Degradation):

Add 2 μl DNAse I (2KU/ml) 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 25ºC).

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 RP and W1 contain irritants. Wear gloves when handling these buffers.

Protocol

Sample Preparation

- Cut off 100 mg of fresh plant tissue or 50 mg of dry plant tissue.
- Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.

Step 1 Lysis

- Add **1 ml of RP Buffer** and **10 μl of ß-mercaptoethanol** to the sample in the mortar and grind the sample until it is completely dissolved.
- Transfer the dissolved sample to a RNase-free 1.5 ml microcentrifuge tube. Incubate at 70ºC for 30 minutes. (invert the tube every 10 minutes)
- Centrifuge at 2-8ºC at 14-16,000 x g for 10 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Step 2 RNA Binding

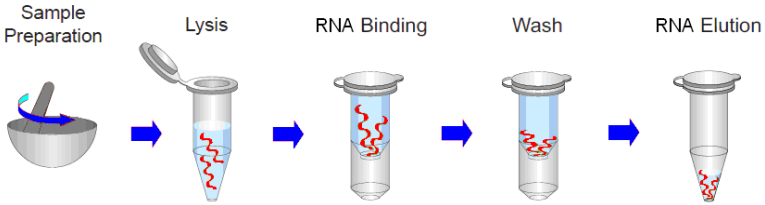
- Add a ½ volume of Isopropanol to the sample from Step 1 and **shake vigorously** (e.g. add 250 μl of Isopropanol to 500 μl of sample).
- Place a **RP Column** in a **2 ml Collection Tube**.
- Transfer the sample mixture to the **RP Column**. Centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through and transfer the remaining mixture to the same **RP Column**.
- Centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through and place the **DP Column** back in the **2 ml Collection Tube**.

Step 3 Wash

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

Step 5 Elution

- To elute RNA, place the **RP Column** in a new RNase-free 1.5 ml microcentrifuge tube.
- Add 50-200μl **REL Buffer** to the center of each **RP Column**, let stand for 2 minutes, and centrifuge at 14-16,000 x g for 2 minutes.



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.