Total RNA Isolation Kit (Plant)

www.fairbiotech.com

For research use only

Sample: 100 mg of fresh plant tissue or 25 mg of dry plant tissue

Yield : up to 30μg

Introduction

The Total RNA Isolation 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 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 60 minutes.

Kit Contents

Catalog No.	RP0004	RP0100	RP0200	RP0300
RP Buffer	4 ml	110 ml	105 ml×2	105 ml×3
W1 Buffer	2 ml	45 ml	85 ml	125 ml
W2 Buffer (Add Ethanol)	300μl×2 (1.2 ml)×2	15 ml (60 ml)	25 ml (100 ml)	25 ml×2 (100ml)×2
REL Buffer	1 ml	10 ml	20 ml	30 ml
RP Columns	4 pcs	100 pcs	200 pcs	300 pcs
Collection Tubes	4 pcs	100 pcs	200 pcs	300 pcs

Quality Control

In accordance with FairBiotech's ISO-certified Total Quality Management System, the quality of the Total RNA Isolation 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.

Total RNA Isolation Kit 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

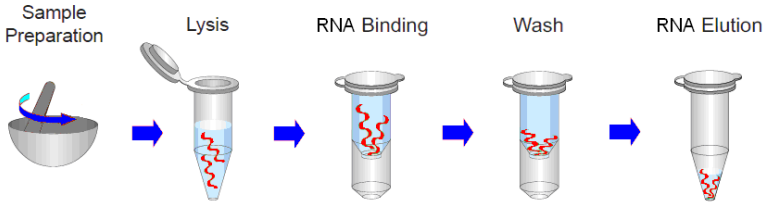
- Add a ½ volume of Isopropanol to the sample from Step 1 and **shake vigorously** (e.g. add 250 μl of Isopropanol to 500 μl of sample).
- Place a **RP Column** in a **2 ml Collection Tube**.
- Transfer the sample mixture to the **RP Column**. Centrifuge at 14-16,000 x g for 30 seconds.
- Discard the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