Total RNA Isolation Kit (Plant)

Cat No. PDC07-0100
Size: 100 Reactions

Sample: 100 mg of fresh plant tissue or 25 mg of dry plant tissue
Format: Spin column
Operation time: within 1 hour
Elution volume: 50~200 µl
Yield: up to 30 µg

Description

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 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

Contents	PDC07-0100	PDC07-0100S
Buffer RP	110 ml	4 ml
Buffer W1	45 ml	2 ml
Buffer W2 (Add ethanol)	15 ml (60 ml)	300 µl x2 (1.5 ml x2)
Buffer RE	10 ml	1 ml
Columns RP	100 pcs	4 pcs
Collection Tubes	100 pcs	4 pcs

Feature

- Delivering 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

Quality Control

The quality of the Dual Genomic DNA Isolation Kit is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Ethanol (96~100%)
- 14.3 M ß-mercaptoethanol
- For Optional Step (DNA Residue Degradation): Add 2 µl of DNase I (2 KU/ml) mixed 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
- For Paraffin-Embeded Tissue: xylene, absolute ethanol
- RNase-free pipet tips and 1.5 ml microcentrifuge tubes
- Liquid nitrogen

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Total RNA Isolation Kit (Plant) Protocol

Step 1 Sample Preparation

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

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 a RNase-free 1.5 ml microcentrifuge tube.
3. Incubate at 75°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.

Step 3 RNA Binding

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

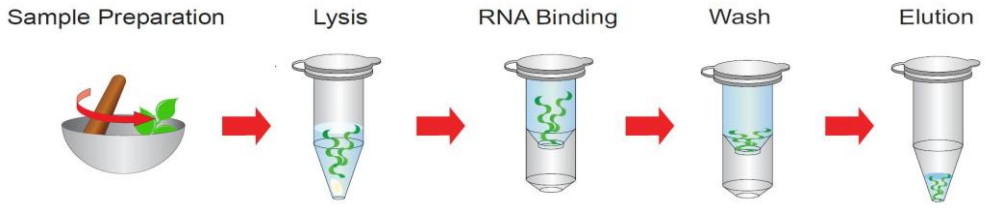
Step 4 Wash

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

Step 5 Elution

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

#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 purifying total RNA 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, EX: RNase Inhibitor.
Low yields of RNA	Incomplete lysis and homogenization	Use the appropriate method for the lysate preparation based on the amount of the starting materials immersed in the Buffer RP to achieve the optimal lysis.
	Incorrect elution conditions	Add 50 µl of the Buffer RE to the center of each Column RP, 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 Buffer W2.

Caution

- During the operation, always wear the latex or vinyl gloves while handling reagents and RNA samples to prevent the RNase contamination.
- Buffers RP and W1 contain irritants. Wear gloves when handling these buffers.
- Add 60 ml of the ethanol to the Buffer W2 before use.
- Check Buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.