Total RNA Isolation Kit (Plant)

Cat. No.: SN020-0100 Size: 100 Reactions
Cat. No.: SN020-0004 Size: 4 Reactions
Sample: 100 mg of fresh plant tissue or 50 mg of dry plant tissue
Format: Spin column
Operation time: within 60 minutes
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 total RNA isolation 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 isolated total RNA is suitable for a variety of routine applications including RT-PCR, Northern Blotting, cDNA Synthesis and Mapping. The entire procedure can be completed within 60 minutes.

Features

- Delivers high-quality total RNA with the fast procedure, ready-to-use RNA for high performance in any downstream application.
- Consistent RNA yield from a small amount of the starting material.

Applications

- RT-PCR.
- Real-time RT-PCR.
- Northern blotting.

Kit Contents

Contents	SN020-0100	SN020-0004
Buffer RP	110 ml X 1 bottle	2 ml X 2 vials
Buffer W1	45 ml X 1 bottle	2 ml X 1 vial
Buffer W2 (add ethanol)	15 ml X 1 bottle (60 ml X 1 bottle)	300 µl X 2 vials (1.2 ml X 2 vials)
Buffer RE	10 ml X 1 bottle	1 ml X 1 vial
RP Column	50 pieces X 2 bags	4 pieces X 1 bag
Collection Tube	50 pieces X 2 bags	

Quality Control

The quality of the Total RNA Isolation Kit (Plant) is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Liquid nitrogen
- Mortar and pestle
- 14.3 M ß-mercaptoethanol
- RNase-free pipet tips and 1.5 ml microcentrifuge tubes
- Absolute ethanol
- Water bath/ Dry bath
- Isopropanol

Buffer Preparation

For Optional Step (DNA Residue Degradation): Add 2 µl of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM MnCl2, 50 µg/ml BSA at 25°C} to the final elution sample. Let it stand for 10 minutes at room temperature.

Total RNA Isolation Kit (Plant) Protocol

Step 1 Sample Preparation

1. Cut off fresh plant tissue (up to 100 mg) or collect 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 an RNase-free 1.5 ml microcentrifuge tube.
3. Incubate at 70°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

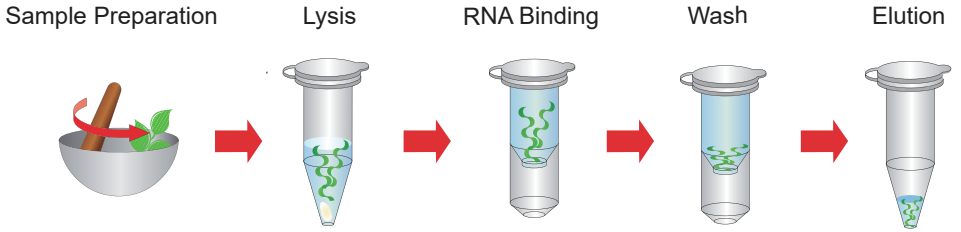
1. Add the half volume of isopropanol to the sample from Step 1 and shake vigorously (e.g. add 250 µl of isopropanol to 500 µl of sample).
2. Place a RP Column in a 2 ml Collection Tube.
3. Transfer the sample mixture to the RP Column.
4. Centrifuge at 14-16,000 x g for 30 seconds.
5. Discard the flow-through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

Step 4 Wash

1. Add 400 µl of Buffer W1 into the RP Column.
2. Centrifuge at 14-16,000 x g for 30 seconds.
3. Discard the flow-through and place the RP Column back into the same 2 ml Collection Tube.
4. Add 600 µl of Buffer W2 (ethanol added) into the RP Column.
5. Centrifuge at 14-16,000 x g for 30 seconds.
6. Discard the flow-through and place the RP Column back into the same 2 ml 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 RP Column in a new RNase-free 1.5 ml microcentrifuge tube.
2. Add 50-200 µl of Buffer RE to the center of each RP Column, let it stand for 2 minutes, and centrifuge at 14-16,000 x g for 2 minutes.
3. Optional Step (DNA Residue Degradation): 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 you did RNA isolation 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, e.g: RiboIN 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 50-200 µl of the Buffer RE to the center of each RP Column, let it stand for 2 minutes, and centrifuge at 14-16,000 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.

Related Ordering information

Cat. No.	Description	Size
SA001-0500	AGAROSE Tablet, 0.5g	100 Tablets
SL001-1000	Novel Juice Supplied in 6X Loading Buffer	1 ml
SD010-R600	1 Kb DNA Ladder RTU	600 µl
ST040-4000	100 mM dNTP Set	4 x 1 ml
SM701-0050	Oligo$(dT)_{20}$ primer	50 µl
SM305-0050	GScript First-Strand Synthesis Kit	50 Reactions
SM306-0050	GScript One-Step RT-PCR Kit	50 Reactions
SR001-2500	RiboIN RNase Inhibitor	2500 U

Caution

- During the operation, always wear the latex or vinyl gloves while handling reagents and samples to prevent the RNase contamination.
- Check Buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- The Buffers RP and W1 contain irritants, so please wear gloves when handling these buffers.
- When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
- When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to the each vial of the Buffer W2, and shake before use.
- All products are for research use only.