PR Reagent (Plant Total RNA Isolation Kit)

Cat. No.: SN007-0100 Size: 100 Reactions
Cat. No.: SN007-0004 Size: 4 Reactions
Sample: Up to 100 mg of fresh plant tissue
Up to 50 mg of dry plant tissue
Format: Reagent form
Operation time: 120 minutes
Release volume: 50~100 µl

Description

The PR Reagent provides an easy 3-step method to isolate the total RNA from plant samples. This unique reagent system ensures the total RNA with a high yield and good quality from the most common plant samples as well as samples high in polysaccharides. If a larger sample is required, the kit volume can be scaled up proportionately, making the kit not only user-friendly but also highly versatile. The RNA phenol extraction is not required, and the entire procedure can be completed in 2 hours. The total RNA (up to 80 µg for fresh plant tissue) is ready for use in RT-PCR, Northern Blotting, cDNA Synthesis and Mapping.

Features

- Fast procedure delivering high-quality total RNA.
- Ready-to-use RNA for high performance in any downstream application.
- Consistent RNA yield from the starting material with a small amount Provides sufficient reagents and 3 steps to treat samples.

Applications

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

Kit Contents

Contents	SN007-0100	SN007-0004
PR buffer 1	100 ml X 1 bottle	2 ml X 2 vials
PR buffer 2	10 ml X 1 bottle	0.5 ml X 1 vial

Quality Control

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

Required Materials

- Mortar and pestle
- RNase-free H_2O
- Chloroform
- Isopropanol
- Microcentrifuge tubes (RNase free)
- ß-mercaptoethanol
- 70% ethanol in H_2O (RNase free)
- Water bath / Dry bath

PR Total RNA Isolation Kit Protocol

Sample Preparation

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

Step 1 Lysis

1. Add 1 ml of the PR buffer 1 and 12 µl of the ß-mercaptoethanol to the sample in the mortar and grind the sample until it is completely dissolved.
2. Transfer the dissolved sample to a 1.5 ml microcentrifuge tube.
3. Incubate at 70°C for 50 minutes.
4. Incubate at 15~30°C for 5 minutes.
5. Centrifuge at 2~8°C at 14-16,000 x g for 15 minutes.
6. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Step 2 Phase Separation

1. Add a 1/10 volume of the PR buffer 2 and 500 µl of the chloroform to the supernatant from the Step 1.
2. Shake vigorously and then centrifuge at 2~8°C at 14~16,000 x g for 10 minutes.
3. Carefully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube.
4. Repeat the Phase Separation Step until the interphase becomes clear, and then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.

NOTE: The number of repetitions is dependent on the sample type, e.g. dense tissue samples may require a higher number of repeats.

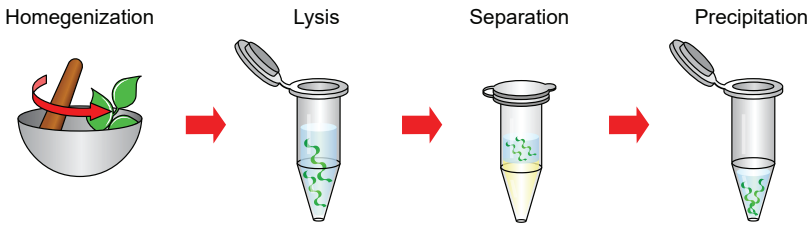
Step 3 RNA Precipitation

1. Add 500 µl of the isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from the Step 2.
2. Mix the sample by inverting gently and incubating on the ice for 10 minutes.
3. Centrifuge at 2~8°C at 14~16,000 x g for 15 minutes.
4. Discard the supernatant and wash the pellet with 1 ml of the 70% ethanol.
5. Centrifuge at 2~8°C at 14~16,000 x g for 5 minutes.
6. Completely discard the supernatant and re-suspend the pellets in 50~100 ul of the RNase-free H_2O.

I7. ncubate for 10 minutes at 60°C to dissolve the pellet.

NOTE:

- Due to the presence of RNase, wear gloves at all times.
- Use sterile, disposable plasticware and automatic pipettes reserved for the RNA work to prevent cross-contamination with RNases.
- Treat non-disposable glassware and plastic-ware before use to ensure that it is RNase-free.



Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did RNA isolation with the kit.

Problem	Cause	Solution
Difficult to dissolve RNA	Incomplete removal of the ethanol	Remove ethanol in the hood briefly.
Genomic DNA containment	Incomplete removal of genomic DNA	DNase treatment.
Degraded RNA / low integrity	RNases contaminant	Work RNases free: Clean everything, use barrier tips, wear gloves and a lab coat. Use RNase-free enzymes and RNase inhibitor.
	Improper sample handling from harvest to lysis	If not processed immediately, freeze the tissue immediately after harvesting, and store it at -80°C or in the liquid nitrogen.
	Tissue highly rich in RNases Incomplete lysis and homogenization	Add RNase inhibitors/inactivators to protect the RNA from degradation, or use a larger volume of the PR buffer 1.
Low yields of RNA	Incomplete lysis and homogenization	Complete homogenization.
	Poor quality of the starting material	Be sure to use the fresh sample and process immediately after collection or freezing the sample at –80°C or in the liquid nitrogen immediately after harvesting.
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA	Remove ethanol in the hood briefly.

Related Ordering Information

Cat. No.	Description	Size
SL001-1000	Novel Juice (Supplied in 6X Loading Buffer)	1 ml
SD010-R500	1 Kb DNA Ladder RTU	500 µl
ST040-4000	100 mM dNTP Set	4 x 1 ml
ST046-1000	100 mM dNTP Set	4 x 250 µl
SM305-0050	GScript First-Strand Synthesis Kit	50 Reactions
SM306-0050	GScript One-Step RT-PCR Kit	50 Reactions

Caution

- Check buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- All products are for research use only.