Plant Total RNA Isolation Kit

Cat No. PDR04-0100(S)
Size: 100 Reactions

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 proportionately, making the kit not only very 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.

Kit Contents

Contents	PDR04-0100	PDR04-0100S
PR buffer 1	100 mL	4 ml
PR buffer 2	15 mL	500 µl

Background on RNA Isolation and Purification

Plants are diverse, and individual species and organs or plant tissues can behave differently during the RNA extraction (and DNA) for use in the molecular studies. Problems encountered include the presence of a large quantity of polysaccharides, high RNase level, various kinds of phenolics, including tannins, low nucleic acid concentrations (high water content), and tissues, such as lignin (wood). Those are difficult to break up, affect DNA and RNA qualities, and inhibit downstream reactions. We offer the isolation and purification kits for the total plant RNA. Therefore we have optimised purification reagent for high quality total plant RNA. The developed protocols enable the study of gene expression by RT-PCR for a large number of samples.

Feature

- 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

Application

- Molecular weight and size of DNA
- Purity of DNA required
- Downstream DNA applications
- Time flexibility
- Ease of DNA extraction technique or method
- Expense reduction

Required Sample of Plant Tissue

Cell	Required Sample
Fresh plant tissue	100 mg
Dry plant tissue	50 mg

Required Materials

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

Protocol

Sample Preparation

1. Cut off 100 mg of the fresh plant tissue or 50 mg of the dry plant tissue.
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 xg 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.

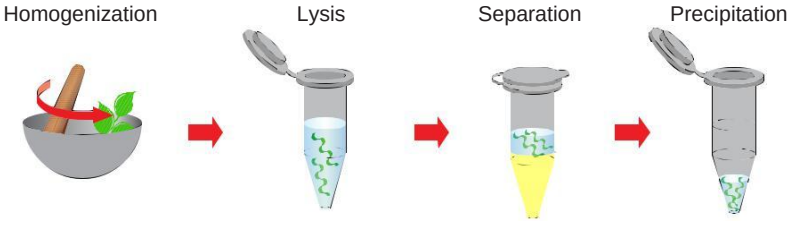
Step 3 RNA Precipitation

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 xg for 15 minutes.
4. Discard the supernatant and wash the pellet with 1 ml of the 70% EtOH.

5. Centrifuge at 2~8°C at 14~16,000 xg for 5 minutes.
6. Completely discard the supernatant and re-suspend the pellets in 50~100 µl of the RNase-free H_2O.
7. Incubate 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 purifying plant total RNA with the kit.

Problem	Cause	Solution
Difficult to dissolve RNA	Incomplete removal of the EtOH	Remove EtOH in the hood briefly.
Genomic DNA containment	Incomplete removal of gDNA	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. Frozen samples must remain frozen until the Lysis Buffer was added. Perform the lysis quickly after adding the Lysis Buffer.
	Tissue highly rich in RNases	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.
		Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the PR Buffer 1 to achieve the optimal lysis.
	Incorrect precipitation conditions	Add the RNase-free H_2O (50~100 µl) and incubate for 10 min at 60°C.
	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 EtOH in the hood briefly.

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.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.