PR Reagent (Plant Total RNA Isolation Kit)

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Introduction

The PR reagent provides an easy 3-step method to isolate total RNA from plant samples. This unique reagent system ensures total RNA with high yield and good quality from most common plant samples and also samples high in polysaccharides. When processing a larger sample, the reagent volume can be scaled-up proportionately, making this reagent not only very user friendly but also highly versatile. 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

Catalog No.	PR0004	PR0050	PR0100
PR Reagent 1	4 ml	50 ml	100 ml
PR Reagent 2	500 µl	8 ml	15 ml

Quality Control

In accordance with FairBiotech's ISO-certified Total Quality Management System, the quality of the FB PR Reagent (Plant Total RNA Isolation Kit) is tested on a lot-to-lot basis to ensure consistent product quality.

Additional requirements

∗mortar and pestle ∗microcentrifuge tubes (RNase free) ∗RNase-free H_2O ∗ß-mercaptoethanol ∗chloroform ∗absolute EtOH for preparing 70% EtOH in H_2O (RNase free) ∗isopropanol

Optional requirements

For complete DNA 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).

PR Reagent (Plant Total RNA Isolation Kit) Protocol

NOTE: Check buffers before use for salt precipitation. Re-dissolve any precipitate by warming to 37°C.

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

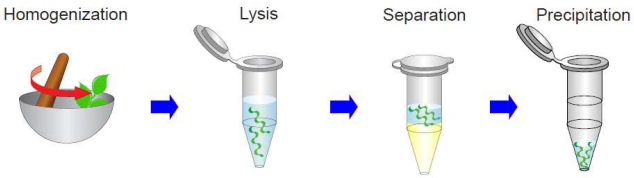
- Add **1 ml of PR reagent 1** and 12 µl of ß-mercaptoethanol to the sample in the mortar and grind the sample until it is completely dissolved. Transfer the dissolved sample to a 1.5 ml microcentrifuge tube.
- Incubate at 70ºC for 50 minutes. (invert the tube every 10 minutes)
- Incubate at 15-30ºC for 5 minutes and then centrifuge at 2-8ºC at 14-16,000 x g for 15 minutes.
- Transfer the supernatant to a new 1.5 ml microcentrifuge tube. Add a 1/10 volume of PR reagent 2 to the supernatant.

Step 2 Phase Separation

- Add 500 µl of chloroform to the supernatant from Step 1.
- Shake vigorously and then centrifuge at 2-8ºC at 14-16,000 x g for 10 minutes.
- Carefully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube.
- Repeat the Phase Separation Step until the interphase becomes clear then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube. (The number of repetitions is dependent on sample type; e.g. dense plant tissue samples may require a higher number of repeats.)

Step 3 RNA Precipitation

- Add 500 µl of isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from step 2.
- Mix the sample by inverting gently and then incubate on ice for 10 minutes.
- Centrifuge at 2-8ºC at 14-16,000 x g for 15 minutes. Discard the supernatant and wash the pellet with 1 ml of 70% EtOH.
- Centrifuge at 2-8ºC at 14-16,000 x g for 5 minutes. Completely discard the supernatant and re-suspend the pellets in 50-100 µl of RNase-free H_2O. Incubate for 10 minutes at 60ºC to dissolve the pellet.



Troubleshooting

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 contamination	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 liquid nitrogen. Frozen samples must remain frozen until the Lysis Buffer is 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.
RNA containment	Incomplete removal of the RNase	RNase A treatment
Low yields of RNA	Incomplete lysis and homogenization	Complete homogenization.
		Cut plant samples into smaller pieces and ensure the pieces are completely immersed in PR Buffer 1 to achieve the optimal lysis.
	Incorrect precipitation conditions	Add RNase-free H2O (50~100µL) and incubate for 10 min at 60°C.
	Poor quality of the starting material	Be sure to use the fresh samples 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.