

# Plant RNabsolute

*For research use only*

- Sample** : 100 mg of fresh plant tissue
- Operation time** : 120 minutes
- Efficiency** : High yield RNA ideal for Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Northern Blotting

**Geneaid**



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## Introduction

Plant RNabsolute 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. If a larger sample is required, the reagent volume can be scaled proportionately, making this reagent not only very user friendly but also highly versatile. DNA 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.

## Quality Control

The quality of Plant RNabsolute is tested on a lot-to-lot basis by isolating total RNA from 100 mg fresh plant tissue samples. A minimum of 20 µg of total RNA is quantified with a spectrophotometer and checked by electrophoresis.

### Kit Contents

Name	RPR004	RPR100
Plant RNabsolute*	4 ml	100 ml
Plant RNabsolute 2*	500 µl	15 ml

\*If the reagents contain sediment, incubate at 65°C for 10 minutes to dissolve.

### Order Information

Product Name	Reactions	Cat. No.
Reagent Genomic DNA Kit	100/1000 rxns	GE100/01K
DNabsolute	100 rxns	NR100
Plant DNabsolute	100 rxns	GR100
RNabsolute	100 rxns	RAR100
Plant RNabsolute	100 rxns	RPR100
96-Well DNabsolute	4/10 x 96 rxns	NRP04/10
96-Well Plant DNabsolute	4/10 x 96 rxns	GRP04/10

## Caution

The components contain irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

## Additional requirements

mortar and pestle, microcentrifuge tubes (RNase free), RNase-free H<sub>2</sub>O, β-mercaptoethanol, chloroform, absolute EtOH for preparing 70% EtOH in H<sub>2</sub>O (RNase free), isopropanol

## Optional requirements

If a larger sample volume is required, scale the Plant RNabsolute proportionately. For complete DNA Degradation, add 2 µl of DNase I (2 KU/ml), mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM MnCl<sub>2</sub>, 50 µg/ml BSA at 25°C} to the final sample in the RNA Precipitation Step. Let stand for 10 minutes at room temperature.

## Plant RNabsolute Protocol

Tissue Dissociation	<ul style="list-style-type: none"> <li>● Cut off 100 mg of fresh plant tissue or 50 mg of dry plant tissue.</li> <li>● Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.</li> </ul>
Step 1 Lysis	<ul style="list-style-type: none"> <li>● Add <b>1 ml of Plant RNabsolute</b> and 12 µl of β-mercaptoethanol to the sample in the mortar and grind the sample until it is completely dissolved.</li> <li>● Transfer the dissolved sample to a 1.5 ml microcentrifuge tube.</li> <li>● Incubate at 70°C for 50 minutes.</li> <li>● Incubate at 15-30°C for 5 minutes.</li> <li>● Centrifuge at 2-8°C at 14-16,000 x g for 15 minutes.</li> <li>● Transfer the supernatant to a new 1.5 ml microcentrifuge tube.</li> </ul>
Step 2 Phase Separation	<ul style="list-style-type: none"> <li>● Add a <b>1/10 volume of Plant RNabsolute 2</b> and 500 µl of chloroform to the supernatant from Step 1.</li> <li>● Shake vigorously and then centrifuge at 14-16,000 x g for 5 minutes.</li> <li>● Carefully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube.</li> </ul> <p><b>Repeat the Phase Separation Step until the interphase becomes clear by adding only 500 µl of chloroform (per repetition) to the upper phase in the new 1.5 ml microcentrifuge tube. Once the interphase becomes clear, transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.</b></p> <p>NOTE: The number of repetitions is dependent on sample type; e.g. dense tissue samples may require a higher number of repeats.</p>
Step 3 RNA Precipitation	<ul style="list-style-type: none"> <li>● Add 500 µl of isopropanol to the 1.5 ml microcentrifuge tube containing the upper phase from step 2.</li> <li>● Gently invert the tube 3-5 times.</li> <li>● Incubate on ice for 10 minutes.</li> <li>● Centrifuge at 14-16,000 x g for 15 minutes.</li> <li>● Discard the supernatant and wash the pellet with 1 ml of 70% EtOH.</li> <li>● Centrifuge at 2-8°C at 14-16,000 x g for 5 minutes.</li> <li>● <b>Completely discard the supernatant</b> and add 50-100 µl of RNase-free H<sub>2</sub>O to the 1.5 ml microcentrifuge tube.</li> <li>● Incubate for 10 minutes at 60°C to dissolve the pellet.</li> </ul>

## Troubleshooting

Problem	Possible Reasons/Solution
Incomplete Lysis	<p><b>Too much sample was used</b></p> <ul style="list-style-type: none"> <li>● Reduce sample volume or separate into multiple tubes and grind the sample completely</li> </ul>
Low Yield	<p><b>Precipitate was formed at Step 3 RNA Precipitation</b></p> <ul style="list-style-type: none"> <li>● Reduce the sample material</li> <li>● Increase incubation time following isopropanol addition to improve total RNA precipitation</li> <li>● Avoid RNase contamination</li> </ul>