Total RNA Isolation Kit (Plant)

Cat. No.:	YTP100	YTP500
Product Name:	Total RNA Isolation Kit (Plant)	
Reactions:	100	500
Sample:	Fresh Plant Tissue	
Yield:	Up to 80 μg for 100 mg of Fresh Plant Tissue	
Format:	Reagent	
Operation:	Centrifuge	
Operation Time:	Within 120 Minutes	

Introduction

Total RNA Isolation Kit (Plant) enables 3-steps operations to isolate total RNA from various plant samples. The scalable purification procedure gently removes contaminants and inhibitors and allows large-volume samples to be purified for use as long-term references. The unique TP Buffer system ensures total RNA with high yield and good quality from most common plant samples and samples high in polysaccharides.

If a larger sample is required, the buffer volume can be scaled proportionately. DNA phenol extraction is not required. The entire procedure can be completed in 2 hours. The total RNA purified with Total RNA Isolation Kit (Plant) is ready for use in a wide range of applications, such as RT-PCR, Northern Blotting, cDNA Synthesis and Mapping.

Features

1. Convenient, scalable purification procedure
2. Reproducible recoveries for constant results.
3. Ready-to use RNA within 2 hours.

Contents

ITEM	YTP100	YTP500
TP1 Buffer*	100ml	500ml
TP2 Buffer*	15ml	75ml

* If there is sediment formed in the buffer, incubate at 65℃ for 10 minutes to dissolve it.

Applications

Purified RNA is ready for direct use in RT-PCR, Real-Time PCR, Northern Blot Analysis, mRNA Selection, Microarrays, cDNA synthesis.

Quality Control

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

Caution

Buffers contain irritant agents. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Additional Requirements:

RNase-free microcentrifuge tubes, absolute ethanol for preparing 70% ethanol in RNase-free water, Chloroform, Isopropanol, mortar and pestle, ß-mercaptoethanol, RNase-free water.

Notes:

If a larger sample volume is required, scale the TP1 Buffer proportionately. For complete DNA Degradation, add 2µl 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 25ºC} to the final sample in the RNA Precipitation Step. Let stand for 10 minutes at room temperature.

Protocol

Tissue Dissociation	★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 TP1 Buffer** 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. ★Incubate at 15-30ºC for 5 minutes. ★Microcentrifuge at 13,000rpm at 2-8ºC for 15 minutes. ★Transfer the supernatant to a new 1.5 ml microcentrifuge tube and add **TP2 Buffer which is 1/10 volume of the supernatant.**
Step 2 Isolation	★Add 500 µl of chloroform to the mixture from Step 1. ★Shake vigorously and then centrifuge at full speed for 5 minutes. ★Carefully remove the upper layer and transfer it to a new 1.5 ml microcentrifuge tube (repeat the Isolation Step until the interphase becomes clear).
Step 3 RNA Precipitation	★Carefully transfer the supernatant to a new 1.5 ml microcentrifuge tube containing 500 µl of Isopropanol. ★Gently invert the tube 3-5 times. ★Incubate on ice for 10 minutes. ★Centrifuge at full speed for 15 minutes. ★Discard the supernatant and wash the pellet with 1 ml of 70% ethanol. ★Centrifuge at 2-8ºC at full speed for 5 minutes. ★**Completely discard the supernatant** and add 50-100 µl of RNase-free water to the 1.5 ml microcentrifuge tube. ★Incubate for 10 minutes at 60ºC to dissolve the pellet.