Ver: 23121

Phytolyze Reagent

Cat.No.: ORN03

Introduction

Phytolyze Reagent is a quick and convenient reagent for use in the extraction of total RNA from plants. A suitable single-step liquid phase separation results in the extraction of RNA.

This is one of the most effective methods for extracting total RNA and can be completed in only 1 hour starting with fresh tissue or cells. The resulting RNA is intact with little or no contaminating DNA and protein. Phytolyze Reagent maintains the integrity of the RNA due to highly effective inhibition of RNase activity while disrupting cells and dissolving cell components during sample homogenization. This RNA can be used for Northern blots, mRNA isolation, *in vitro* translation, RNase protection assay, cloning and polymerase chain reaction (PCR).

Reagents Required but Not Provided

- Chloroform
- 100% Isopropanol
- 75% Ethanol
- RNase-free water

Procedures

1. Place 100mg fresh or frozen sample in a 1.5mL microcentrifuge tube.
   Note: Samples may be frozen by dipping them in liquid nitrogen.
   Samples should be finely chopped before transferring to the microcentrifuge tube.
2. Add 700µL Phytolyze Reagent to the tube containing sample.
3. Add 20µL β-mercaptoethanol to each tube.
4. Vortex for 10 to 20 seconds to mix, make sure to disperse all clumps and then incubate for 30 minutes at 65°C, vortex the tube for several times during incubation.
5. Add 700µL chloroform, mix by inverting the tube for several times, Centrifuge for 10 minutes at 12,000 rpm (~13,400 ×g).
6. Pipette the supernatant to a new tube, add 700µL ice-cold isopropanol, and mix by inverting the tube several times. Allow the sample to stand for 5–10 minutes at room temperature. Centrifuge at 12,000 rpm (~13,400 ×g) for 10 minutes at 2-8°C. The RNA precipitate will form a pellet on the bottom of tube.

7. Remove the supernatant and wash the RNA pellet by adding minimum of 1mL of 75% ethanol per 700µL of Phytolyze Reagent. Vortex the sample and then centrifuge at 12,000 rpm (~13,400 ×g) for 5 minutes at 2-8°C.
   Note: Samples can be stored in ethanol at 2-8°C for at least 1 week and up to 1 year at –20°C.
8. Briefly dry the RNA pellet for 5–10 minutes by air drying or under a vacuum. Do not let the RNA pellet dry completely, as this will greatly decrease its solubility. Do not dry the RNA pellet by centrifugation under vacuum. Add an appropriate volume of formamide, or RNase-free water to the RNA pellet. To facilitate dissolution, mix by repeated pipetting with a micropipette at 55–60°C for 10–15 minutes.
   Optional (but recommended): DNase I treatment (Cat. No.: ORT411, not provided) for enzymatic removal of residual genomic DNA after dissolving the RNA pellet in an appropriate volume of formamide, or RNase-free water.

Troubleshooting Guide

A. Low yield may be due to:
   - Less amount of initial sample.
   - Incomplete homogenization or lysis of samples.
   - The final RNA pellet may not have been completely dissolved.

B. If the A_{260}/A_{280} ratio is <1.65:
   - The amount of sample used for homogenization may have been too small.
   - The final RNA pellet may not have been completely dissolved.

C. If there is degradation of the RNA:
   - The tissues may not have been immediately processed or frozen after collection.
   - The samples used for isolation or the isolated RNA preparations may have been stored at –20°C instead of –70°C as specified in the procedure.
   - Aqueous solutions or tubes used for procedure may not have been RNase-free.
   - Formaldehyde used for the agarose gel electrophoresis may have had a pH value < 3.5.

Storage

Store the product at room temperature.
For long-term storage, keep at 4°C.