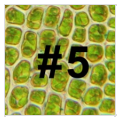


Aug 20, 2019

# RNA Isolation from Plant Tissue Protocol 5: pBIOZOL Method

1 Works for me dx.doi.org/10.17504/protocols.io.4rdgv26

Scott C. Edmunds
GigaScience/BGI Hong Kong/Bauhinia Genome

ABSTRACT

Implemented by: Beijing Genomics Institute

This protocol is part of a collection of eighteen protocols used to isolate total RNA from plant tissue. (RNA Isolation from Plant Tissue Collection: https://www.protocols.io/view/rna-isolation-from-plant-tissue-439gyr6)

journal.pone.0050226.s011.PDF

MATERIALS TEXT

## Reagents

- 5 M NaCl
- Chloroform
- Isopropyl alcohol
- 75 % ethanol (DEPC treated)
- pBIOZOL Reagent (Beijing Bai billion New Technology Co., Beijing, China)
- RNase-free water

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

1 Grind tissue to a powder in liquid nitrogen.

2 Add **1.3 ml** of cold ( **4 °C**) pBIOZOL Reagent for up to **100 mg** of frozen, ground tissue.

2.1 Mix by briefly vortexing or flicking the bottom of the tube until the sample is thoroughly suspended.

3 Incubate the tube for **00:05:00** at **20 °C**.

Lay the tube down horizontally to maximize surface area during RNA extraction.

4 Centrifuge for **00:02:00** at **12000 x g**.

4.1 Transfer the supernatant to an RNase-free tube.

5 Add **100 µl** of **5 Molarity (M)** NaCl to the extract.

5.1 Tap tube to mix.

6 Add **300 µl** of chloroform.

6.1 Mix thoroughly by inversion.

7 Centrifuge the sample at **4 °C** for **00:10:00** at **12000 x g** to separate the phases.

7.1 Transfer the top aqueous phase to an RNase-free tube.

8 Add to the aqueous phase an equal volume of isopropyl alcohol.

8.1 Mix.

8.2 Let stand at **20 °C** for **00:10:00** .

9 Centrifuge the sample at **4 °C** for **00:10:00** at **12000 x g**.

10 Decant the supernatant, taking care not to lose the pellet.

10.1 Add **1 ml** of chilled 75 % ethanol to the pellet.

Pellet may be difficult to see.

11 Centrifuge at **Room temperature** for **00:05:00** at **12000 x g**.

11.1 Decant the liquid carefully, taking care not to lose the pellet.

11.2 Briefly centrifuge to collect the residual liquid and remove it with a pipette.

12 Add **10 µl** – **30 µl** RNase-free water to dissolve the RNA.

12.1 Pipette the water up and down over the pellet to dissolve the RNA.

If any cloudiness is observed, centrifuge the solution at **Room temperature** for **00:01:00** at **12000 x g** and transfer the supernatant to a fresh tube.