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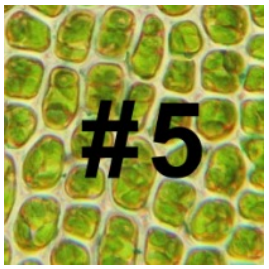
RNA Isolation from Plant Tissue Protocol 5: pBIOZOL Method



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In 1 collection

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Protocol status: Working
These protocols were used for RNA extraction from plant tissues in order to support the One Thousand Plants initiative's work to produce RNA-Seq transcriptomes from a diverse collection of plant samples.

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Protocol Integer ID: 25093

Keywords: RNA, RNA isolation, RNA extraction, plant tissue

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)

Attachments

journal.pone.0050226...

281KB

Materials

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 .

> Note
>
> 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 [M] 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.

Note

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.

Note

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.