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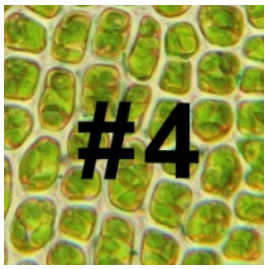
RNA Isolation from Plant Tissue Protocol 4: CTAB-PVP-TRIzol Method



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Peer-reviewed method

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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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

PDF

journal.pone.0050226...

277KB

Materials

MATERIALS

TRIzol Reagent **Thermo Fisher Scientific** **Catalog #**15596026

Troubleshooting

Safety warnings

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

1 Grind tissue to a powder in liquid nitrogen.

2 Add 200 mg – 500 mg of ground tissue to 3.0 mL of pre-heated extraction buffer in a 5 ml tube.

3 Vortex the tube until the tissue is mixed with the buffer.

4 Incubate the tube at 65 °C for 00:30:00, vortexing briefly (00:00:15) every 2–3 min during the incubation.

5 Aliquot the mixture into four 2 ml RNase free tubes, 1 mL in each tube.

6 Spin the tube at 12000 x g for 00:10:00 in a centrifuge.

Note

All of the insoluble matter should form a pellet at the bottom of the tube.

7 Pour the supernatant into a new 2 ml tube.

8 Add an equal volume of 24:1 chloroform:isoamyl alcohol to fill the tube.

9 Vortex tubes until the phases mix and appear cloudy.

9.1 Incubate at 20 °C for 00:05:00 .

10 Spin the tubes at 12000 x g for 00:10:00 in a centrifuge.

11 Transfer the upper aqueous phase to new 2 ml RNase free tubes.

11.1 Repeat steps 8 to 10 one more time. go to step #8

12 Transfer the upper, aqueous phase to new 2 ml RNase free tubes.

12.1 Add 1/3 volume of [M] 10 Molarity (M) LiCl to each tube.

12.2 Mix and let stand at 4 °C for 06:00:00 – 08:00:00 or overnight to precipitate RNA.

13 Spin tubes at 18000 x g for 00:20:00 in a centrifuge and decant the supernatant, taking care not to lose the pellet.

14 Add 1 mL 75 % cooled ethanol to the pellet.

15 Spin the tube at maximum speed for 00:05:00 in a centrifuge.

15.1 Decant the supernatant carefully.

15.2 Repeat steps 14 and 15 one more time. go to step #14

16 Open cap and air-dry the pellet.

17 Add 30 µL RNase free water to dissolve the pellet.

17.1 Then add 300 µL TRIzol reagent and equal volume of chloroform to TRIzol reagent (Invitrogen).

17.2 Vortex vigorously and store at 20 °C for 00:05:00 .

18 Centrifuge at > 12000 x g for 00:10:00 .

19 Transfer the upper, aqueous phase to a new 2 ml RNase free tube.

19.1 Add 2 volumes of cooled 100% ethanol, 1/10 volume of NaAc and 2 µL of glycogen.

19.2 Mix and incubate at -20 °C for 02:00:00 .

20 Spin tubes at > 12000 x g for 00:20:00 at 4 °C in a centrifuge.

21 Decant the supernatant taking care not to lose the pellet.

21.1 Add 1 mL 75 % ethanol to the pellet.

21.2 Let tube stand at 20 °C for 00:03:00 .

22 Centrifuge at 4 °C for 00:05:00 at > 12000 x g .

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

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

23 Repeat steps 21 and 22 one more time. go to step #21

24 Open cap and air dry the pellet.

25 Add 10 µL – 30 µL RNase free water to dissolve the pellet.