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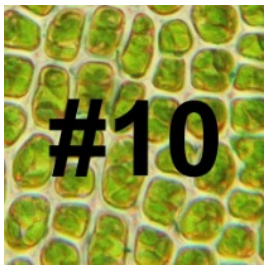
RNA Isolation from Plant Tissue Protocol 10: TRIzol LS Reagent Method



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

Abstract

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This protocol follows the procedures provided with the TRIzol LS Reagent (Invitrogen). TRIzol LS Reagent is a monophasic solution of phenol and guanidine isothiocyanate that can be used in isolation of total RNA from a wide variety of tissues and organisms, in addition to plants. This protocol was used in the isolation of total RNA from some algae samples (see Supplementary Table 1).

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...

287KB

Materials

MATERIALS

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

Safety warnings

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

Before start

Note

All centrifugation steps are performed at 4 °C.

1 Centrifuge at lowest speed to cause algae to form pellet.

1.1 Wash several times with sterile culture medium (not DEPC-treated).

1.2 After washing, the algal material is aliquoted into portions of 250 µL (ca. 50 mg – 100 mg packed cell volume).

2 Homogenize each 250 µL portion of pellet material to a powder in liquid nitrogen using mortar and pestle prechilled with liquid nitrogen.

3 Add 750 µL TRIzol LS to each 250 µL of homogenized algal material.

3.1 Add more nitrogen if needed (see also Procedure described in Protocol 9).

4 Homogenization is continued until the TRIzol is pulverized as well.

5 Thaw and aliquot homogenate into several Eppendorf tubes.

6 Add 50 µL potassium acetate ([M] 0.2 Molarity (M) final concentration) to each sample.

7 Incubate for 00:05:00 at 20 °C .

8 Add 200 µL chloroform (for polysaccharide-rich algae), or 100 µL BCP to each sample.

8.1 Shake samples for 00:00:15 .

9 Incubate at 20 °C for 00:10:00 .

10 Centrifuge samples at 12000 x g for 00:15:00 .

11 RNA will remain in the upper, aqueous phase (ca. 70 % of the applied TRIzol).

12 Carefully transfer each RNA phase into RNase-free 1.5 ml tubes.

13 Add 500 µL isopropanol.

14 Incubate for 01:00:00 at -20 °C .

15 Centrifuge at 12000 x g for 00:10:00 .

16 Wash pellet with 75 % ethanol.

17 Gently suspend pellet in solution.

18 Centrifuge at 7500 x g for 00:05:00 .

19 Repeat ethanol wash steps. go to step #17

20 Dry pellet at 50 °C for 00:05:00 – 00:10:00

Note

Appearance of drying pellet is important: drying should be terminated when the pellet begins to become transparent; contaminated RNA remains white)

21 Add RNAse-free water.

21.1 Incubate at 55 °C – 60 °C for 00:10:00 .

22 Dissolve pellet completely by pipetting.