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

1 Works for me dx.doi.org/10.17504/protocols.io.4rwgv7e



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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>)

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MATERIALS

NAME ▾

CATALOG # ▾

VENDOR ▾

TRIzol Reagent

15596026

Thermo Fisher Scientific

MATERIALS TEXT

Reagents

- Chloroform or BCP (1-bromo-3-chloropropane)
- Isopropanol
- 75 % Ethanol (in DEPC-treated water)
- Potassium acetate
- TRIzol LS Reagent (Invitrogen)
- RNase-free water

SAFETY WARNINGS

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

BEFORE STARTING



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 (**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



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.



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