

Aug 20, 2019

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

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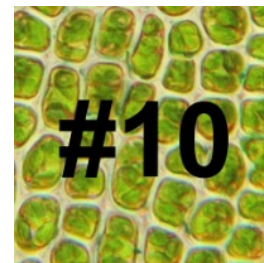
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[dx.doi.org/10.17504/protocols.io.4rwgv7e](https://dx.doi.org/10.17504/protocols.io.4rwgv7e)

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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.**

**Created:** June 26, 2019

**Last Modified:** August 23, 2019

**Protocol Integer ID:** 25110

**Keywords:** RNA isolation, RNA extraction, RNA, plant tissue, rna isolation from plant tissue protocol, rna isolation from plant tissue collection, total rna from plant tissue, isolation of total rna, rna isolation, plant tissue protocol, total rna, trizol ls reagent, rna, trizol ls reagent method, plant tissue collection, bryophyte, algae sample, plant, trizol, algae, guanidine isothiocyanate, addition to plant

## Abstract

Implemented by: Michael Melkonian and Barbara Surek (algae) and Juan Carlos Villarreal (bryophytes)

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



[journal.pone.0050226...](#)

287KB


## Materials

### MATERIALS

 TRIZOL Reagent Thermo Fisher Scientific Catalog #15596026


## Troubleshooting

## 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  $\mu$ L (ca.  50 mg –  100 mg packed cell volume).
- 2 Homogenize each  250  $\mu$ L portion of pellet material to a powder in liquid nitrogen using mortar and pestle prechilled with liquid nitrogen.
- 3 Add  750  $\mu$ L TRIzol LS to each  250  $\mu$ L 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  $\mu$ L potassium acetate (  0.2 Molarity (M) final concentration) to each sample.
- 7 Incubate for  00:05:00 at  20  $^{\circ}$ C .
- 8 Add  200  $\mu$ L chloroform (for polysaccharide-rich algae), or  100  $\mu$ L 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.