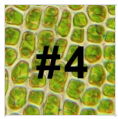

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RNA Isolation from Plant Tissue Protocol 4: CTAB-PVP-TRIzol Method

1 Works for me dx.doi.org/10.17504/protocols.io.4q5gvy6

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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)

journal.pone.0050226.s011.PDF

MATERIALS

NAME	CATALOG #	VENDOR
TRIzol Reagent	15596026	Thermo Fisher Scientific

MATERIALS TEXT

Reagents

CTAB-PVP buffer:
- CTAB (2 % w/v)
- PVP-40 (2% w/v)
- 100 mM Tris-HCl (pH 8.0)
- 25 mM EDTA
- 2 M NaCl
- Spermide (0.5 g/L), (Warmed to 65 °C in a water bath to suspend in solution)
- Add β-ME to final concentration of 2 % before use

SSTE buffer:
- 1 M NaCl
- SDS (0.5 % w/v)
- 10 mM Tris-HCl (pH 8.0)
- 1 mM EDTA

Other Reagents:
- 75 % ethanol (DEPC treated)
- 100 % ethanol
- Acid phenol (pH 4.5)
- Chloroform
- Isoamyl alcohol
- 10 M LiCl
- Glycogen (5 mg/ml)
- 3 M NaAc (pH 5.2)
- TRIzol reagent (Invitrogen)
- 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 200 mg – 500 mg of ground tissue to 3 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.

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.