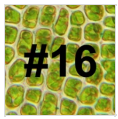

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RNA Isolation from Plant Tissue Protocol 16: CTAB-Hot Acid Phenol Method for Algae

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

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ABSTRACT

Implemented by: Falicia Goh and Neil Clarke

This RNA isolation method is a combination and modification of the hot acid phenol method (protocol 14) and that described by Asif et al[6]. This method was used for two taxa (P. cruentum and B. braunii).

#14 Protocol 14: Ambion Trizol RNA Extraction in Microcentrifuge Tubes with Turbo DNAfree Digestion
by Julia Roßmanith

PREVIEW RUN

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)

[6] Asif, M.H., Dhawan, P. & Nath, P. A simple procedure for the isolation of high quality RNA from ripening banana fruit. Plant Molecular Biology Reporter 18, 109-115 (2000).

journal.pone.0050226.s011.PDF

MATERIALS TEXT

Reagents

Extraction Buffer:

- 100 mM Tris-HCl pH 8.2
- 1.4 M NaCl
- 2 % CTAB
- 20 mM EDTA pH 8.2
- 1 µl of 2-mercaptoethanol per ml of buffer just before use
- DEPC treated water

The final reaction buffer was filter purified using Nalgene 0.22 µM filter.

Other reagents:

- Acid phenol (pH 4.3)
- Phenol:chloroform (5:1) acid equilibrated to pH 4.7 from Sigma
- Chloroform
- Isopropanol
- 70 % ethanol (diluted in DEPC treated water H_2O)

- 3 M Sodium acetate pH 5.5
- 3 M Lithium chloride

SAFETY WARNINGS

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

1 Preheat phenol and phenol:chloroform to **65 °C**.

Heated phenol should not be re-used.

2 Collect algae cells via centrifugation for **00:10:00** at **16100 x g** at **Room temperature**.

2.1 Flash freeze pellets with liquid nitrogen and keep at **-80 °C** until extractions are carried out.

3 Re-suspend the frozen pellet in **800 µl** of preheated extraction buffer.

4 Incubate at **65 °C** for **01:00:00** . Gently vortex every **00:15:00** .

5 Cool to **Room temperature**.

5.1 Add equal volume of chloroform.

5.2 Shake vigorously until 2 phases form an emulsion.

6 Collect the aqueous phase by centrifuging for **00:10:00** in micro-centrifuge at **16100 x g** at **Room temperature**.

7 Collect aqueous phase and re-extract with an equal volume of chloroform.

7.1 Centrifuge as above.

8 Collect aqueous phase and add [M]**10 Molarity (M)** LiCl to a final concentration of [M]**3 Molarity (M)**.

8.1 Allow the RNA to precipitate at **4 °C** overnight.

9 Recover the RNA by centrifugation at **16100 x g** at **4 °C** for **00:20:00**.

10 Dissolve pellet in DEPC treated water.

10.1 Extract once with hot acid phenol.

11 Extract the aqueous phase with equal volume of phenol:chloroform (5:1).

12 Vortex for **00:01:00** at **Room temperature**.

12.1 Spin for **00:05:00** in a micro-centrifuge at top speed.

13 Extract the aqueous phase with equal volume of chloroform.

14 Collect aqueous phase and add 1/30 volume of [M]**3 Molarity (M)** sodium acetate pH 5.5 and 0.1 volume of 100 % ethanol.

14.1 Mix well and keep on ice for **00:30:00**.

14.2 Centrifuge in cold for **00:25:00**.

14.3 A white jelly-like pellet consisting mostly of polysaccharides is obtained and discarded.

15 To the clear supernatant add [M]**3 Molarity (M)** sodium acetate pH 5.2 to a final concentration of [M]**0.3 Molarity (M)** and 3 volumes of 100 % ethanol.

15.1 Allow the RNA to precipitate at **-80 °C** for **03:00:00** to overnight.

16 Spin in micro-centrifuge at **4 °C** at top speed for **00:20:00** .

17 Wash the pellet with 70 % ethanol.

18 Invert tubes and air dry at room temperature.

19 Resuspend pellets in **50 µl** of DEPC treated water.