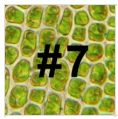

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RNA Isolation from Plant Tissue Protocol 7: pBIOZOL-LiCl Method

1 Works for me dx.doi.org/10.17504/protocols.io.4rvgv66


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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 TEXT

Reagents

- Acid phenol (pH 4.5)
- Chloroform
- Isopropyl alcohol
- 75 % ethanol (DEPC treated)
- 100 % ethanol
- 2 M NaAc (pH 4.2)
- 3 M NaAc (pH 5.2)
- 5 M NaCl
- 10 M LiCl
- pBIOZOL Reagent (Beijing Bai billion New Technology Co., Beijing, China)
- RNase-free water

SSTE Buffer:

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

SAFETY WARNINGS

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

1 Grind tissue to a powder in liquid nitrogen.

2 Add **1.3 ml** of cold (**4 °C**) pBIOZOL reagent for up to **100 mg** gram of frozen, ground tissue.

2.1 Mix by briefly vortexing or flicking the bottom of the tube until the sample is thoroughly suspended.

3 Incubate the tube for **00:05:00** at **Room temperature**.

Lay the tube down horizontally to maximize surface area during RNA extraction.

4 Centrifuge at **12000 x g** for **00:02:00** .

5 Transfer the supernatant to a new 1.5 ml RNase-free tube.

6 Add **50 µl** of **2 Molarity (M)** NaAc (pH 4.2) to the extract.

6.1 Tap tube to mix.

6.2 Then add **100 µl** **5 Molarity (M)** NaCl and **300 µl** chloroform.

6.3 Vortex vigorously.

7 Centrifuge the mixture at **4 °C** for **00:10:00** at **12000 x g** to separate the phases.

7.1 Transfer the top aqueous phase (about **400 µl** – **500 µl**) to a new 1.5 ml RNase-free tube.

8 Add to the aqueous phase 1/3 volume of **10 Molarity (M)** LiCl.

8.1 Mix and let stand at **4 °C** overnight.

9 Centrifuge the mixture at 4 °C for 00:20:00 at > 12000 x g.

10 Decant the supernatant, taking care not to lose the pellet.

10.1 Add 1 ml of 75 % ethanol to the pellet.

10.2 Stand the tube at Room temperature for 00:03:00 .

Pellet may be difficult to see.

11 Centrifuge at 4 °C for 00:03:00 at > 12000 x g.

11.1 Decant the liquid carefully, taking care not to lose the pellet.

11.2 Briefly centrifuge to collect the residual liquid and remove it with a pipette.

12 Repeat the previous two steps.

13 Add 50 µl RNase-free water to dissolve the RNA pellet.

13.1 Pipette the water up and down over the pellet to dissolve the RNA.

If you extract more than 100 mg plant tissues, combine different extractions to one tube.

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14 Add SSTE buffer to RNA to a total volume of 600 µl.

14.1 Then add equal volume of 25:24:1 acid phenol:chloroform:isoamyl alcohol to the tube.

15 Vortex the tube until the phases mix and appears cloudy.

15.1 Then incubate at **20 °C** for **00:05:00** .

16 Centrifuge at **12000 x g** for **00:10:00** in a microcentrifuge.

17 Transfer the top, aqueous phase to a new 1.5 ml RNase-free tube.

17.1 Add equal volume of 24:1 chloroform:isoamyl alcohol to the tube.

18 Vortex the tube until the phases mix and appear cloudy.

18.1 Then incubate at **20 °C** for **00:05:00** .

19 Centrifuge at **12000 x g** for **00:10:00** .

20 Transfer the top aqueous phase to a new 1.5 ml RNase-free tube.

20.1 Add to the aqueous phase 2 volumes of 100 % ethanol, 1/10 volume of **3 Molarity (M)** NaAc (pH 5.2) and **2 µl** **5 mg/ml** glycogen.

20.2 Invert tube to mix.

20.3 Store at **-20 °C** for **02:00:00** .

21 Centrifuge at **4 °C** for **00:20:00** at > **12000 x g**.

21.1 Decant the supernatant carefully to avoid losing the pellet.

22 Add **1 ml** of 75 % ethanol to the pellet.

22.1 Incubate at **20 °C** for **00:03:00**.

23 Centrifuge at **4 °C** for **00:05:00** at **12000 x g**.

23.1 Decant the liquid carefully, taking care not to lose the pellet.

23.2 Briefly centrifuge to collect the residual liquid and remove it with a pipette.

24 Repeat step 18 and 19.

25 Open cap and air-dry the pellet no more than **00:05:00**.

26 Add **30 µl** RNase-free water to dissolve the pellet.

27 Before library construction, treat RNA with DNase I.