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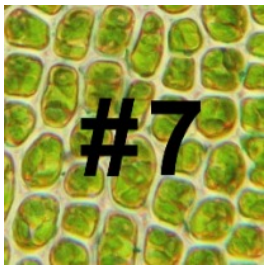
RNA Isolation from Plant Tissue Protocol 7: pBIOZOL-LiCl Method



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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.

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Protocol Integer ID: 25109

Keywords: RNA, RNA isolation, RNA extraction, plant tissue

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)

Attachments

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284KB

Materials

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 .

Note

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 [M] 2 Molarity (M) NaAc (pH 4.2) to the extract.

6.1 Tap tube to mix.

6.2 Then add 100 µL [M] 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 [M] 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 .

Note

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.

> Note
>
> 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 [M] 3 Molarity (M) NaAc (pH 5.2) and 2 µL [M] 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.