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RNA Isolation from Plant Tissue Protocol 13: Trizol/RNAqueous Midi-Kit

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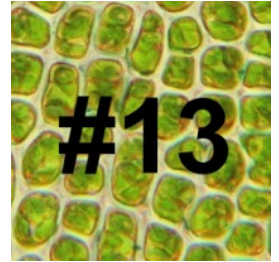
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Protocol status: Working

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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Keywords: RNA, RNA extraction, RNA isolation, plant tissue, rna isolation from plant tissue collection, rna isolation from plant tissue protocol, total rna from plant tissue, rna isolation, rna, total rna, plant tissue protocol, plant tissue collection, rnaqueous midi, trizol method

Abstract

Implemented by: Megan Rolf and Toni M. Kutchan

Note

Note: (two samples from C. dePamphilis and P. Ralph only used the RNAqueous Midi-Kit)

This protocol is based on a combination of two methods: The Trizol method described by Chomczynski and Sacchi⁴ and the Ambion® RNAqueous®-Midi Kit (Life Technologies, Carlsbad, CA), with minor modifications.

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>)

⁴Chomczynski, P. & Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry 162, 156-159 (1987).

Attachments



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

287KB

Materials

MATERIALS

 RNAqueous™-Midi Total RNA Isolation Kit **Thermo Fisher Scientific Catalog #AM1911**

 TURBO DNA-free™ Kit **Thermo Fisher Scientific Catalog #AM1907**

Safety warnings

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



- 1 Quickly weigh out  3 g of frozen tissue and place in a pre-chilled mortar containing liquid nitrogen.
- 2 Grind the sample into fine powder without allowing it to thaw.
- 3 Add the sample into a 50 ml centrifuge tube containing  30 mL of **extraction buffer** (10 ml buffer/g tissue).
- 4 Vortex for  00:01:00 , then incubate at  Room temperature for  00:05:00 .
- 5 Add  6 mL of chloroform (2 ml chloroform/g tissue) and shake vigorously for at least  00:00:20 .
- 6 Centrifuge at  3095 x g for  00:20:00 at  4 °C .
- 7 Move supernatant to new 50 ml tube. Add one volume isopropanol (about  22.5 mL) to precipitate RNA.
- 8 Invert gently several times. Then incubate at  Room temperature for  00:10:00 .
- 8.1 Invert gently several times.
- 8.2 Then incubate at  Room temperature for  00:10:00 .
- 9 Centrifuge at  3095 x g for  00:10:00 at  4 °C .



- 10 Wash pellet with  45 mL of 70 % ethanol. (1/2)
- 11 Centrifuge at  3095 x g for  00:02:00 at  4 °C . (1/2)
- 12 Wash pellet with  45 mL of 70 % ethanol. (2/2)
- 13 Centrifuge at  3095 x g for  00:02:00 at  4 °C . (2/2)
- 14 Air dry pellet for  00:05:00 (if not completely dry, it's still okay to move on to the next step).

15

Note

The following steps use the **RNAqueous Midi Kit from Ambion (AM1911)**.

Dissolve the pellet in  5 mL of lysis/binding Solution.

Note

Heating at  37 °C and vortexing will help dissolve pellet.

- 16 Heat  4.5 mL of elution solution to  100 °C (for later use).

**Note**

Use a 17 X 100 mm round bottom sterile polypropylene tube with loose-fitting dual position cap.

17 Add  5 mL of 64 % ethanol to RNA in lysis/binding solution.

17.1 Draw into a 10 ml syringe through an 18 gauge needle.

18 Remove needle and attach filter unit. Slowly push the lysate/ethanol mixture through.

Note

Often times the filter gets clogged. There are tips in the kit manual for dealing with this but we found these did not help. We tried to get the solution through one filter even with some intense pressure. Sometimes two filters were required and the following wash/elution steps were performed on both filters.

19 After filtering the solution, force air through using a clean 10 ml syringe until no more white foam is expelled (at least 3 or 4 times).

20 Wash with 100 % volume of Wash Solution #1 (using syringe). Use a clean 18 g needle to draw up solution.

21 Force air through a few times again.

22 Wash with 70 % volume Wash Solution #2/3. Repeat once using syringe.

23 Force air through again until no more water droplets or fine spray can be seen.

24 Elute at least 2 times into a 2 ml tube (only use  500 μ L at a time, so elute three times per tube to get about 1.5 ml total) using  100 °C Elution Solution and a sterile 3



ml syringe.

25 LiCl precipitate each sample using the LiCl provided in the kit.

25.1 Add ½ volume of LiCl.

25.2 Place at -20 °C for at least 00:30:00 .

25.3 Centrifuge at maximum speed in a microcentrifuge (about 16000 x g) for 00:15:00 at Room temperature .

25.4 Wash pellet with 1 mL of 70 % ethanol.

25.5 Centrifuge again with the same conditons for 00:05:00 .

25.6 Remove supernatant and air dry pellet.

25.7 Resuspend in 50 µL or 100 µL RNase free H₂O.

Note

Heating and vortexing can help with this.

26 DNase Treatment using the **TURBO DNA-free kit from ambion (AM1907)**



26.1 Add  1.5 μL of DNase + 0.1 volumes of 10X buffer.

26.2 Incubate at  37 °C for  00:25:00 .

26.3 Add another  1.5 μL of DNase.

26.4 Incubate at  37 °C for another  00:25:00 .

26.5 Add 0.1 volumes DNase Inactivation Reagent.

26.6 Incubate  00:05:00 at  Room temperature , flicking tubes every minute.

26.7 Centrifuge at  10000 x g for  00:01:30 .

26.8 Move supernatant to a new tube.

27 LiCl precipitate the supernatant again (same as above).

Note

The final resuspension volume should be between  50 μL –  100 μL using RNase free H_2O .