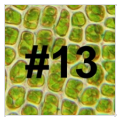

Aug 20, 2019

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

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

Scott C. Edmunds
GigaScience/BGI Hong Kong/Bauhinia Genome

ABSTRACT

Implemented by: Megan Rolf and Toni M. Kutchan

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 Sacchi[4] 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)

[4]Chomczynski, P. & Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanatephenol-chloroform extraction. Analytical Biochemistry 163, 156-159 (1987).

journal.pone.0050226.s011.PDF

MATERIALS

NAME	CATALOG #	VENDOR
RNAqueous™-Midi Total RNA Isolation Kit	AM1911	Thermo Fisher Scientific
TURBO DNA-free™ Kit	AM1907	Thermo Fisher Scientific

MATERIALS TEXT

Reagents

Extraction buffer:

- 0.8 M guanidine thiocyanate
- 0.4 M ammonium thiocyanat,
- 0.1 M sodium acetate pH 5.0
- 5 % glycerol
- 38 % phenol (pH 4.3 -5.0)

Other reagents and equipment:

- 100 % ethanol
- 70 % ethanol
- Chloroform
- Isopropanol, 99.5 % DNase, RNase, and Protease free
- Nuclease free H_2O
- RNAqueous-Midi Kit
- Turbo DNA-free kit
- 18 G ½ needle

- 14 ml polypropylene round-bottom tube
- 10 ml syringe
- 3 ml syringe
- 50 ml centrifuge tube

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

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

Dissolve the pellet in **5 ml** of lysis/binding Solution.

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

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

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.

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 µl** 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 H_2O.

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).

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