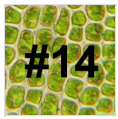

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RNA Isolation from Plant Tissue Protocol 14: Ambion Trizol RNA Extraction in Microcentrifuge Tubes with Turbo DNAfree Digestion

1 Works for me dx.doi.org/10.17504/protocols.io.4u6gwze

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ABSTRACT

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Concise version of Protocol (longer version available on request from Ingrid Jordon-Thaden, ingridejt@gmail.com)

This procedure eliminates the mortar and pestle homogenization of tissues and instead grinds tissue in 2 ml microcentrifuge tubes. The method closely follows Ambion's protocols and could be used in a 96-well format. This method worked great for species that proved to be difficult to extract with other methods (i.e. woody and aquatic plants).

Both the addition of β-mercaptoethanol in extraction and high salts (recommended by Ambion: 0.8 M sodium citrate and 1.2 M NaCl) in precipitation were tried with this method and yield or quality was not affected. The addition of Sarkosyl significantly improved both yield and quality.

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)

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MATERIALS

NAME	CATALOG #	VENDOR
TRIzol Reagent	15596026	Thermo Fisher Scientific
TURBO DNA-free™ Kit	AM1907	Thermo Fisher Scientific

MATERIALS TEXT

Reagents

- 20 % Bleach
- 95 % Ethanol
- RNase Zap
- liquid N_2
- TRIzol Reagent (Ambion, Life Technologies, Carlsbad, CA)
- 20 % Sarkosyl (optional for difficult species)
- 100 % Chloroform
- 100 % Isopropanol
- 75 % Ethanol
- Turbo DNA-free Kit (Ambion, store at -20 °C)
  - 10X Turbo DNAase buffer
  - Turbo DNAase
  - DNAase Inactivation Reagent

Other materials

- Zirconia beads, prebaked at 200 °C for 4 hours
- P1000 RNA-free filter tips
- P100 RNA-free filter tips
- RNAase free pipettes: P1000, P100
- Stretch winter gloves
- Nitrile gloves (to wear over winter gloves, latex will crack in liquid N_2)
- Two large plastic Eppendorf tube racks (96 well)
- Black shaker block (for automatic shaker/pulverizer machine)
- One ice bucket to hold the Eppendorf tube rack on ice
- One small ice bucket with liquid N_2 to hold the black shaker block
- One small ice bucket with liquid N_2 to hold the tubes while weighing
- 24 – 2 ml RNA-free Eppendorf tubes (to freeze the tissue in; note, do not use cheap tubes as they will crack)
- 72 – 1.7 mL RNA-free tubes (3 batches of 24, pre-labeled; 96 tubes if you plan on using the Sarkosyl step, this adds one more chloroform extraction)
- 3x – 50mL Falcon tubes
  - to hold 100 % Chloroform
  - to hold 100 % Isopropanol (also called 2-propanol)
  - to hold 75 % Ethanol

Equipment:

- Automatic shaker for tissue homogenization
- 24-place centrifuge cooled to 4 °C
- Vortexer
- Water bath at 50 °C
- Incubator at 37 °C with orbital shaker

SAFETY WARNINGS

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

BEFORE STARTING

Listed below are two slightly different methods for tissue collection: one directly in microcentrifuge tubes, and one in 50 ml tubes.

Leaf Collection in microcentrifuge tubes (best to use 60 to 100 mg of tissue for high throughput)

Pre-label RNAase free 2 mL tubes, place 5 zirconia beads (pre-baked at **200 °C** for **04:00:00**) in each tube and place in boxes.

Cut leaf tissue and put into the tube, then directly into small cooler with liquid N_2. Store the boxes with the leaves in **-80 °C** freezer.

Leaf collection in 50 mL Falcon tubes (for large collections so many extractions can be made on the same sample if necessary)

Identify the species to collect, give it a collection number, and write the number of the 50 ml Falcon tube. Use a scissor or pin to cut a hole in the top of the tube. Fill the tube with N_2 and place in cooler with N_2 and rack. Clean scissors with Ethanol and RNAzap. Cut the youngest leaf tissue and immediately put into the Falcon tube for freezing. Take a specimen of the plant for a voucher. Place the tubes in the **-80 °C** freezer.

All centrifuge steps at **4 °C** unless otherwise specified.

1 Clean all surfaces and equipment with 20 % bleach, 95 % ethanol, and RNase Zap, and place supplies in hood.

2 Prepare sample tubes with labels.

3 Fill microcentrifuge tubes with 5 zirconia beads (if they have not been done before tissue collection).

4 Prepare aliquots of the following solutions into 50mL Falcon tubes: 75 % ethanol, 100 % chloroform, 100 % isopropanol.

5 Fill dewar with liquid N_2.

6 Get samples from **-80 °C** and place them in the black shaker block that is sitting in a bath of liquid N_2 (if already in microcentrifuge tubes), and check labels.

7 Prepare frozen tissue in microcentrifuge tubes from 50 ml Falcons (if tissue is not in them already).

7.1 Fill two small coolers with liquid nitrogen (one for microcentrifuge tubes, the other for Falcon tubes).

7.2 Doing about 3 or 4 plants at a time, use forceps or a spatula to move **60 mg** to **100 mg** of tissue into the microcentrifuge tubes from the 50 ml Falcon tubes.

7.3 Get a weight for each of the samples, add more if necessary.

7.4 This kit requires **60 mg** to **100 mg** of plant tissue.

If you are doing aquatic plants use 2x the weight since so much of the weight is water.

7.5 Repeat these steps until you have finished all of the samples.

8 Before pulverizing the frozen tissue, check each tube for beads, making sure they are easily moving within the tube.

9 Taking the black shaker block with the 24 samples, place tightly into the automatic shaker, doing this quickly as to not allow thawing.

9.1 Shake for **00:02:00** (they will stay frozen).

10 Place block back into the liquid nitrogen cooler if needed to shake another **00:02:00** .

10.1 Shake a second time after the block appears to be frozen.

10.2 Keep on liquid nitrogen, until TRIzol is added.

11 Add **1 ml** of TRIzol solution.

Before opening the tubes, tapping the tube on the bottom on the bench will empty most of the leaf tissue that is in the lid from the shaking process.
If it does not move, you can use the vortexer with the solution added to force it out of the lid.

Optional adjustment is to add **50 µl** to **100 µl** of 20 % Sarkosyl to each sample with the TRIzol.

12 After each tube has had the TRIzol solution added, vortex immediately, both the top and bottom of the tube, until all tissue is hydrated. Vortexing for **00:02:00** can be common.

13 Then place tube on ice, and do this sequentially until you have all 24 tubes finished.

14 Once the batch is ready, incubate at **Room temperature** for **00:05:00** .

15 Centrifuge at **12000 x g** for **00:10:00** at **4 °C**.

16 Pipette aqueous solution to a new 1.7 ml tube (will be **900 µl** to **800 µl**).

If Sarkosyl is used, be aware it will be a thick, viscous layer at the interface. Try not to pull any into the aqueous layer.

16.1 Add **200 µl** of 100 % chloroform to each tube.

Do not change this volume or more protein will be forced into the aqueous layer.

16.2 Vortex for **00:00:10** – **00:00:15** .

Solution should be milky colored.

16.3 Incubate at **Room temperature** for **00:10:00** .

16.4 Centrifuge at **12000 x g** for **00:15:00** at **4 °C**.

Be careful not to disturb layers.

16.5 Remove upper aqueous layer (should be clear), **500 µl** to **700 µl**, and put in new 1.7 ml tube.

16.6 If you suspect the sample does not look “clean” or if you had used the Sarkosyl addition, repeat steps 16.1 to 16.5 (i.e. the chloroform step).

17 Precipitate and pellet the nucleic acids by adding **500 µl** of isopropanol.

17.1 Mix by inverting the rack.

18 Incubate at **Room temperature** for **00:10:00** .

19 Centrifuge at **12000 x g** for **00:15:00** at **4 °C**.

20 Pour off supernatant as waste.

21 Add **1000 ml** of 75 % ethanol to tube with pellet.

21.1 Vortex until the pellet is loose.

22 Centrifuge at **8900 x g** for **00:05:00** at **4 °C**.

23 Pour off ethanol into beaker and tap tube on tissue to pull as much ethanol off as you can.

24 Centrifuge the “empty” tube for **00:02:00** at **4 °C**.

25 Using pipette, pull off excess ethanol collected at the bottom of the tube.

Final pellet should be clear. If white, then may still have salts and you can repeat the ethanol wash a second time, however, the DNA removal step seems to also remove salt contamination.

26 Let the pellet dry for **00:02:00** at **Room temperature**, but no more than **00:10:00** .

27 Re-dissolve in **50 µl** of RNase-free water.

If the RNA is pure, it should be instant.

27.1 To aid in dissolution, incubate at 55 °C for 00:10:00 in water bath.

Never incubate longer than 00:10:00 at this temperature. Also never increase the temperature, as this can cause RNA degradation. If pellet does not dissolve immediately, store at 4 °C overnight (or until dissolved). However, the best samples dissolve with no trouble.

27.2 Vortex gently when finished.

28 Check on nanodrop for concentration.

Samples should be diluted to be less than [M]200 ng/µl before proceeding to the DNAase steps. However, we have done the following steps with samples that are up to [M]700 ng/µl and had success. We suggest only diluting when you have [M]2000 ng/µl or [M]3000 ng/µl of RNA.

29 Removal of DNA using Turbo DNA-free kit by adding 0.1 volume of 10X Turbo DNasebuffer (usually 5 µl if no dilution of RNA was made) and add 1 µl of Turbo DNase to theRNA (always only 1 µl) and mix gently.

30 Incubate at 37 °C while shaking on the orbiter inside the incubation oven for 00:30:00 .

31 Add resuspended DNase Inactivation Reagent (typically 0.1 volume; 5 µl if no dilution of RNA was made) and mix well (vortex very briefly).

32 Incubate at Room temperature for 00:02:00 , vortexing occasionally.

33 Centrifuge at 10000 x g for 00:01:30 at 4 °C.

33.1 Transfer to a new tube.

34 Measure RNA with nanodrop again.

[M]**100 ng/µl** is ideal, but there will most likely be more. Expect some loss from theTurbo Kit. Also, if the first time the spectra appeared contaminated, this step may have cleaned it some. The OD 260/280 ratio should be 1.8 to 2.2 (not less than 1.6), in order to get good transcriptome library construction.