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RNA Isolation from Plant Tissue Protocol 12: Hot Acid Phenol Method for Angiosperms

1 Works for me dx.doi.org/10.17504/protocols.io.4rxgv7n

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ABSTRACT

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This RNA isolation method is a multi-component method involving an initial extraction by hot acid phenol and then a purification and DNase treatment using the RNeasy Mini Kit by Qiagen. The method described below is a modification of a method described by van Tunen et al.[3].

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)

[3] van Tunen, A.J. et al. Cloning of the two chalcone flavanone isomerase genes from Petunia hybrida: coordinate, light-regulated and differential expression of flavonoid genes. The EMBO Journal 7, 1257-1263 (1988).

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MATERIALS

NAME	CATALOG #	VENDOR
RNase-Free DNase Set	79254	Qiagen
RNeasy® Mini Kit	74104	Qiagen

MATERIALS TEXT

Reagents

Extraction Buffer:

- 100 mM Tris pH 9.0
- 1 % SDS (v/v, starting from 10% SDS stock solution)
- 100 mM LiCl
- 10 mM EDTA
- RNase-free water

All components were filter purified and then the final reaction buffer was also filter purified using a Millipore Stericup.

Other reagents:

- Acid phenol (pH 4.3)
- Chloroform:isoamyl (24:1)
- 70 % ethanol (diluted in RNase free H_2O)
- 4 M LiCl

SAFETY WARNINGS

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

1 Add **3 ml** of saturated acid phenol (pH 4.3) and **3 ml** of RNA extraction buffer to a 15 ml snap cap tube. Warm tube to **65 °C** in a fume hood using a heat block.

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1.2 Warm tube to **65 °C** in a fume hood using a heat block.

2 Homogenize tissue in liquid nitrogen using a mortar and pestle.

3 Transfer up to **1 g** of ground tissue to a tube containing the phenol-buffer mixture and close the snap cap tube completely (second stop). Immediately mix tube by hand. Vortex until the phases mix and appear cloudy. Keep at **Room temperature** or **65 °C** depending on whether SDS is precipitating.

Use a spatula and funnel chilled in liquid nitrogen to transfer the powdered tissue to the tube.

3.1 Transfer up to **1 g** of ground tissue to a tube containing the phenol-buffer mixture and close the snap cap tube completely (second stop).

3.2 Immediately mix tube by hand.

3.3 Vortex until the phases mix and appear cloudy.

3.4 Keep at **Room temperature** or **65 °C** depending on whether SDS is precipitating.

4 Centrifuge at **2500 x g** for **00:10:00** .

5 Transfer aqueous phase to a new 15 ml tube.

6 Add **3 ml** of chloroform:isoamyl (24:1 ratio). Close the snap cap tube completely (second stop) and mix contents immediately by hand. Then vortex until the phases mix and appear cloudy.

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6.2 Close the snap cap tube completely (second stop) and mix contents immediately by hand.

6.3 Then vortex until the phases mix and appear cloudy.

7 Centrifuge at **2500 x g** for **00:10:00** .

8 Transfer aqueous phase to a new 15 ml tube.

9 Precipitate nucleic acids by gently adding **3 ml** of isopropanol to the tube followed by gentle inversion to mix the phases. Precipitate for **01:00:00** at **4 °C**.

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9.2 Precipitate for **01:00:00** at **4 °C**.

10 Centrifuge at **2500 x g** for **00:10:00** .

11 Remove the supernatant without dislodging the pellet.

12 Rinse the pellet with **1 ml** of chilled 70 % ethanol made with RNase-free H_2O.

13 Transfer pellet and ethanol to a new 1.5 ml tube and invert to wash.

14 Centrifuge at **16000 x g** in a microcentrifuge for **00:02:00** and remove the supernatant.

15 Spin dry the RNA pellet by successive removal of any remaining liquid following **00:01:00** centrifugations at maximum speed in a microcentrifuge. Repeat this process until no liquid is seen when the tube is flicked.
Allow the pellet to air dry for **00:01:00** to **00:05:00**, depending on the size of the pellet.

Avoid over-drying since this will negatively affect re-suspension in water.

15.1 Spin dry the RNA pellet by successive removal of any remaining liquid following **00:01:00** centrifugations at maximum speed in a microcentrifuge.

15.2 Repeat this process until no liquid is seen when the tube is flicked.

15.3 Allow the pellet to air dry for **00:01:00** to **00:05:00**, depending on the size of the pellet.

Avoid over-drying since this will negatively affect re-suspension in water.

16 While on ice, re-suspend the pellet in **500 µl** RNase-free H_2O using gentle pipetting.

Ensure the pellet is completely re-suspended before proceeding to step 17.

17 Add **500 µl** of **4 Molarity (M)** LiCl. Precipitate the RNA overnight at **4 °C**.

17.1 Add **500 µl** of **4 Molarity (M)** LiCl. **4 °C**.

17.2 Precipitate the RNA overnight at **4 °C**.

18 Centrifuge at **16000 x g** for **00:30:00** at **4 °C** in a microcentrifuge.

19 Remove supernatant by pipette.

20 Wash the pellet three times with **200 µl** of chilled 70 % ethanol diluted in RNase-free H_2O. Centrifuge at **4 °C**.

Remove the pellet from the wall of the tube to wash the pellet more completely. Pellet will now dislodge easily and care must be taken when removing the supernatant between washes.

21 Spin the pellet. Remove ethanol and dry as in step 15. **go to step #15** Centrifuge at **4 °C**.

21.1 Spin the pellet.

21.2 Remove ethanol and dry as in step 15. **go to step #15**

21.3 Centrifuge at **4 °C**.

22 Dissolve pellet in RNase-free H_2O on ice.

The volume of the water is dependent on the size and clarity of the pellet, as well as the viscosity of the re-suspension, but **50 µl** can be used as a standard starting point.

23 Centrifuge RNA extract for **00:01:00** at **4 °C**.
Check concentration and integrity using a NanoDrop 2000 spectrophotometer.

23.1 Centrifuge RNA extract for **00:01:00** at **4 °C**.

23.2 Check concentration and integrity using a NanoDrop 2000 spectrophotometer.

24 Purify and DNase treat up to **100 µg** of RNA on a RNeasy Mini Kit spin column (pink) by Qiagen.

Follow the 'RNA Cleanup' protocol as published in the RNeasy Handbook, including the on-column DNase digestion with the RNase-Free DNase Set by Qiagen.

25 Elute RNA twice from the column using 40 µl of 95 °C RNase-free water to a total of 80 µl.

However, if the mass of RNA applied to the column is less than 50 µg, then use only 40 µl to elute.