# Protocol

TD-P Revision 1.0

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# Plant RNA Extraction Protocol

## Introduction

Extracting good quality RNA with sufficient yield is critical for downstream applications, such as cDNA synthesis, gene amplification, and RT-qPCR. This protocol is a modified SDS-LiCl method, as reported by Vennapusa et al. 2020. We selected this protocol for RNA plant extraction because it was tested on different tissues such as mature, developing, and germinated seeds, as well as leaves and roots exposed to various abiotic stresses. These plant tissues are rich in starch and other secondary metabolites, making it harder to obtain high-yield and quality RNA. Furthermore, this protocol was tested for plants which are difficult to extract RNA: like wheat, corn, and sorghum. Read our GoldBio article to find out more about why it is so difficult to extract plant RNA.

## Materials

### Plant material

Seeds (mature, developing, or germinated), leaves, and roots exposed to different abiotic stresses can be used as plant material for this RNA protocol. Furthermore, this protocol has been tested on cereals (such as wheat, corn, and sorghum), obtaining both high yield and quality RNA.

#### Buffer and Stock solutions

**0.1% DEPC water per 1000 mL:**

- 1 mL DEPC (diethyl pyrocarbonate) reagent
- 1000 mL of Milli-Q water
- Mix on a magnetic stir plate to dissolve the DEPC. Leave the solution mixing overnight.
- Autoclave at 121°C for 15 minutes to inactivate the DEPC.

**1M Tris-HCl per 1000 mL:**

- 121.14 g Tris-HCl
- 800 mL of 0.1% DEPC-treated water
- Adjust to pH 8.0 with 1M HCl
- Complete the volume up to 1000 mL with 0.1% DEPC-treated water
- Store at room temperature

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**0.5M EDTA Disodium per 1000 mL:**

- 186.1 g of EDTA Disodium
- 800 mL of 0.1% DEPC-treated water
- Adjust to pH 8.0 with 5M NaOH
- Fill to 1000 mL with 0.1% DEPC-treated water
- Store at room temperature

**20% SDS per 100 mL:**

- 20 g of SDS (sodium dodecyl sulfate)
- 80 mL of 0.1% DEPC-treated water
- Mix on a heated stir plate at 60°C until complete dissolution
- Fill to 100 mL with 0.1% DEPC-treated water
- Store at room temperature

**3M Sodium acetate per 100 mL:**

- 24.6 g of sodium acetate
- 50 mL of 0.1% DEPC-treated water
- Adjust to pH 4.0 with 1M HCl
- Fill to 100 mL with 0.1% DEPC-treated water
- Store at room temperature

**Phenol: Chloroform: Isoamyl alcohol (25:24:1) per 50 mL:**

- 25 mL of phenol saturated with 0.1M citrate buffer
- 24 mL of chloroform
- 1 mL of isoamyl alcohol
- Prepare just before start the RNA extraction

**80% ethanol per 100 mL:**

- 80 mL of absolute ethanol
- 20 mL of autoclaved 0.1% of DEPC-treated water
- Mix well and store at 4°C.

**RNA extraction buffer per 100 mL:**

- 10 mL of 1M Tris-HCl
- 5 mL of 0.5M EDTA Disodium
- 2.5 g of PVP (Polyvinyl pyrrolidone PVP10)
- 14.61 g of NaCl
- Mix with moderate heating of 40℃
- Fill to 97.5 mL with 0.1% DEPC-treated water
- Autoclave at 121°C for 15 minutes
- Add 2.5 mL of β-Mercaptoethanol just before starting the RNA extraction.

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### Individual Reagents

- PVP (Polyvinyl pyrrolidone PVP10)
- NaCl
- Chloroform
- LiCl (lithium chloride)

### Supplies and Equipment

- Ceramic mortar and pestle
- Glassware
- Micro-spoon or spatula
- DNase/RNase free microcentrifuge tubes
- DNase/RNase free pipet tips
- Tube racks
- Fume hood chamber
- Magnetic stirrer/ with heat
- pH meter
- Vortex
- Autoclave
- Centrifuge
- Gel electrophoresis
- Nanodrop

## Method

1. Pretreat all the plastic, glassware, mortar and pestle with 0.1% DEPC-treated, Milli-Q water and autoclave.

2. Similarly, prepare all the solutions with autoclaved DEPC-treated water.

3. On the RNA extraction day, have a checklist with all the materials and solutions ready in a clean and aseptic place. It can be on a UV-sterilized flow hood chamber. Always use gloves and RNase-DNase free tips and plastics.

4. Collect fresh plant material.

   If your sample cannot be processed for RNA extraction immediately after harvest, freeze the plant tissue in liquid nitrogen and store it at -80°C. Usually, a volume of 1:5 (tissue to liquid nitrogen) is ideal. Let the tissue get completely frozen on liquid nitrogen. Once frozen, use tweezers to transfer the tissue to a 50 mL falcon tube that has been previously chilled to -80°C.

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5. Grind the tissue using a mortar and pestle. First, add a volume of liquid nitrogen to the mortar, then add the tissue and quickly grind the tissue with the help of a previously chilled pestle. Do not let the tissue thaw. Work quickly to avoid thawing and add additional liquid nitrogen as needed.

6. Pre-chill a mini spoon or spatula (test what works best for you) and the microcentrifuge tubes on liquid nitrogen.

7. Transfer about 100 mg of plant tissue with the spoon/spatula to the microcentrifuge tube.

8. Immediately add 600 µL of RNA extraction buffer.

9. Gently vortex to mix.

10. Add 60 µL of 20% SDS.

11. Gently vortex to mix and centrifuge at 16,000 x g for 5 minutes at 4°C.

12. Carefully collect the upper aqueous phase using RNase/DNase free pipet tips.

13. Add an equal volume of Phenol:Chloroform:Isoamyl alcohol (25:24:1) to the vial.

14. Gently vortex to mix and centrifuge at 16,000 x g for 5 minutes at 4°C.

15. Carefully collect the upper aqueous phase using RNase/DNase free pipet tips.

16. Add 200 µL of chloroform.

17. Gently vortex to mix and centrifuge at 16,000 x g for 5 minutes at 4°C.

18. Transfer the upper aqueous phase to a new microcentrifuge tube.

19. Add 160 µL of 8M LiCl and 60 µL of 3M Sodium acetate.

20. Incubate for 15 minutes at -20°C.

21. Centrifuge at 16,000 x g for 5 minutes at 4°C.

22. Remove the supernatant and wash the pellet with 2M LiCl

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23. Centrifuge again at 16,000 x g for 5 minutes at 4°C.

24. Remove the supernatant and add pre-chilled 80% ethanol to wash the pellet.

25. Centrifuge again at 16,000 x g for 5 minutes at 4°C.

26. Discard the supernatant and let dry the ethanol at room temperature. Be careful to not overdry the pellet.

27. Finally, add 100 µL of autoclaved DEPC-treated water and store at -80°C.

## Measurement of the extracted RNA quality and quantity

## Nanodrop

1. Take out the tubes containing the extracted RNA from the freezer and let thaw slowly on ice.

2. Gently mix the extracted RNA via 10 µL pipet.

3. Add 1 µL of the solution on the Nanodrop.

4. Measure the absorbance for A260/A230 and A260/A280. Ratios of ≥2 (A260/A230) and 1.8 (A260/A280) for each absorbance would suggest high quality RNA. The Nanodrop will also calculate the amount of extracted RNA in µg/µL.

### Gel electrophoresis running conditions

1. Clean a 250 mL beaker by adding 20 mL of deionized water. Heat for 90 seconds in a microwave, then discard any remaining water.

2. Weight 1 g agarose and add 100 mL of 1X TAE to the 250 mL beaker.

3. Heat in a microwave for up to 90 seconds (for larger volumes, heat up to 99 seconds) or until agarose boils. Do not let the agarose solution boil over.

4. Allow the agarose to cool to about 50°C (10- 15 minutes) and add 3 µL of Gel Red.

5. Pour the agarose slowly into an electrophoresis gel box and let the agarose solidify. If case bubbles form, they can be pushed away using a small pipet tip.

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6. In a small tube, add 2 µL of loading buffer to 4 µL of each RNA sample and briefly mix by pipet. Load the RNA samples into the gel.

7. Run for 20-30 minutes at 137 volts.

8. Read in the trans-illuminator.

9. You should be able to visualize two bands corresponding to the 28S and 18S rRNA. For total RNA, the first band is about 4000 bp for 28s subunit, for 18s subunit is about 1500 bp. Bands are separated about 2000 bp. Two bands is an indication of good integrity RNA.

## Associated Products

- Diethylpyrocarbonate (DEPC) (GoldBio Catalog # D-340)
- Tris HCl (GoldBio Catalog # T-095
- EDTA Disodium, dihydrate (GoldBio Catalog # E-210)
- Agarose LE (GoldBio Catalog # A-201)
- 1 kb PLUS™ DNA Ladder (GoldBio Catalog # D011)

## References

Vennapusa, A.R., Somayanda, I.M., Doherty, C.J. et al. A universal method for high-quality RNA extraction from plant tissues rich in starch, proteins and fiber. Sci Rep 10, 16887 (2020). https://doi.org/10.1038/s41598-020-73958-5