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Plant RNA purification using TRIzol (TRI reagent) V.2

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Protocol status: Working

We use this protocol and it's working

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Abstract

Extraction of total RNA from plant tissue using TRIzol (or TRI reagent) followed by DNA nuclease treatment.

Attachments



[TRIreagent_SDS.pdf](#)

196KB



[chloroform_SDS.pdf](#)

134KB

Guidelines

TRI reagent and chloroform are hazardous - handle with care, operate in fume hood, wear vinyl gloves and safety glasses.

Materials

- TRIzol (or TRI reagent)
- Chloroform (or chloroform : IAA [24:1])
- Isopropanol
- 80% Ethanol
- Nuclease-free H₂O (e.g. 0.01% DEPC-treated H₂O) or Tris-EDTA (10 mM Tris-Cl, pH 6.5, 0.1 mM EDTA)
- 2 mL safe-lock Eppendorf tubes
- 1.5 mL microcentrifuge tubes
- Tissue lyser or mortar and pestle
- RNase-Free DNase Set (Qiagen)
- RNA loading dye, 2X (NEB)

Troubleshooting



Safety warnings

- ! TRI reagent - hazardous
- Chloroform - hazardous

Ensure you read SDS documents (attached) and organise appropriate waste vessels (fume hood).


Before start

Ensure benches and equipment are RNase free.

For RNase-Free DNase Set (Qiagen): Prepare DNase I stock solution by dissolving the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase-free water. Mix gently by inverting the vial. Divide into single use aliquots and store at -20 °C. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.


RNA purification

- 1 Collect 50-100 mg of plant tissue and freeze immediately in liquid N₂.
- 2 Grind tissue to fine powder under liquid N₂ using tissue lyser or mortar + pestle, then immediately add 1 mL TRI reagent (1 mL per 100 mg tissue).



Note, achieving a fine grind is critical to high yields of intact RNA.
- 3 Invert each tube by hand ~20x and incubate at room temperature for 5 minutes (DO NOT vortex samples as it may result in RNA degradation).
- 4 Add 1/5 volume of pre-mixed chloroform:isoamyl alcohol (24:1), cap tubes, shake vigorously (by hand) for 15 seconds (solution should become cloudy), then incubate at room temperature for 3 minutes.
- 5 Centrifuge at 14,000 rcf for 10 minutes at 4°C.
- 6 Transfer the upper aqueous phase to a new microfuge tube (approx. 400-600 µL).
- 7 Repeat steps 4 and 5 (approx. 300-400 µL).

Note, if you are observing buffer and salt carryover in your purified RNA (high 230 nm absorbance), reduce volume of upper-phase recovered.
- 8 (Optional) If the expected RNA concentration is ≤10 µg/mL, add 1/10 volume of 3M NaOAc (pH 5.5) and/or 100 µg/mL GlycoBlue (or glycogen).


- 9 Add equal volume of 100% isopropanol, then mix by inverting tubes ~20x by hand.
- 10 Incubate at -20°C for 1 hour. Alternatively, incubate overnight to capture small RNAs.
- 11 Centrifuge samples at 14,000 rcf for 20 minutes at 4°C.
- 12 Remove the supernatant, you should observe a white pellet.



- 13 Add 1 mL of 80 % ethanol and invert tube ~10x.
- 14 Centrifuge samples at 10,000 rcf for 5 minutes at room temperature.
- 15 Remove supernatant, carefully since the pellet often becomes dislodged at this step.
- 16 Air-dry pellet at room temperature for 5 minutes.
- 17 Resuspend pellet in RNase-free water (e.g. 0.01% DEPC-treated water) or Tris-EDTA (10 mM Tris-Cl, pH 6.5, 0.1 mM EDTA).

DNA nuclease treatment and ethanol precipitation

- 18 Make up volume of RNA solution to 87.5 μ L with nuclease-free water. This can be performed with an aliquot or total sample from the previous step.
- 19 Add 10 μ L Buffer RDD and 2.5 μ L DNase I stock solution (Qiagen RNase Free DNase Set) and mix with gentle pipetting.
- 20 Incubate at room temperature for 5-10 minutes.
- 21 Add 500 μ L of 100% ethanol.
- 22 (Optional) Add 3 μ L glycogen or GlycoBlue, and 10 μ L NaOAc (pH 5.5) to aid RNA precipitation.
- 23 Mix by gentle inversion.
- 24 Incubate samples for at least 1 hour at -20 $^{\circ}$ C (overnight, if purifying small RNAs).





- 25 Centrifuge at 14,000 rcf at 4 °C for 20 minutes.
- 26 Remove supernatant and rinse pellet with 1 mL of 80% ethanol.
- 27 Centrifuge at 10,000 rcf for 5 minutes at 4 °C.
- 28 Remove supernatant without disturbing pellet and air-dry for 2 minutes.
- 29 Resuspend pellet in RNase-free water (e.g. 0.01% DEPC-treated water) or Tris-EDTA (10 mM Tris-Cl, pH 6.5, 0.1 mM EDTA).

Quality control

- 30 Take 50-100 ng aliquot of RNA and mix 1:1 with 2X RNA loading dye (NEB).
- 31 Incubate RNA at 65 °C for 5 minutes.
- 32 Load and run samples on a 1% agarose TBE gel.
- 33 Nanodrop RNA to check for purity and quantity.