PALL Laboratory

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Protocol

Protocol for Total RNA Purification from Plant Leaves Using Pall Nucleic Acid Binding Nanosep® Centrifugal Device

1. Consumables and Reagents

Table 1

Consumables for Total RNA Purification (nuclease-free consumables are recommended)

Supplier	Product Description	VWR Cat. No.
Pall Laboratory	Nucleic Acid Binding (NAB) Nanosep Centrifugal Device	76360-454, 76360-456
VWR	Ethanol (not denatured)	71001-866
VWR	Spectrophotometer Cuvettes ~100 µL (260/280 nm)	47743-840
VWR	Tubes 15 mL (RNase-DNase free)	89401-574
VWR	Tubes 50 mL (RNase-DNase free)	89401-572
VWR	Microcentrifuge tubes 1.5 mL (RNase-DNase free)	76005-210
VWR	Needles (20) (0.9 mm)	89219-312
VWR	Syringe (10 mL)	89215-218

Table 2

Reagents for Total RNA Purification (nuclease-free reagents are recommended)

Supplier	Product Description	VWR Cat. No.
Various	Proteinase K	N/A
Various	DNaseI Set	N/A
Various	Lysis Buffer	N/A
Various	Wash Buffer 1	N/A
Various	Wash Buffer 2	N/A
Various	Nuclease-free water	N/A
Various	TE Buffer (pH 7.5)	N/A
VWR	Tris Buffer pH 7.0 (1 M)	89500-584
VWR	Lysozyme	97062-136
VWR	DTT (25 g)	97063-758

2. Instruments

- Microcentrifuge
- Spectrophotometer
- Vortex
- Pestle and mortar
- Liquid nitrogen

3. Important Points Before Starting

- Clean all equipment/material to be used for RNA extraction.
- All centrifugation steps are performed at room temperature at 10,000 – 14,000 x g.
- It is essential to work quickly and efficiently when working with RNA.
- Only use the receiver tubes provided in the box. There are enough provided to complete the below process. All Buffers should be allowed to equilibrate to room temperature before use.
- Briefly centrifuge tubes after vortexing to remove drops from inside the lid.
- Change pipette tips between all liquid transfers. Pall recommends use of sterile RNA-free pipette tips.

4. Harvest and Pre-Lysis of Cells

- The amount and type of material being processed will result in variances in yield.
- Determine the amount of tissue to be processed by weighing. Do not use more than 100 mg.
- Place plant leaves in liquid nitrogen and immediately grind to a powder with a pestle and mortar.
- Decant powdered tissue into an RNase-free, liquid nitrogen cooled tube (not provided) and allow the liquid nitrogen to evaporate. Do not allow the tissue to thaw and follow the below steps.

5. Protocol

1. Add 350 µL of lysis buffer (with added DTT) to the powdered sample.
2. Using a syringe and needle, pull the lysate into the syringe and press on the plunger to release the lysate back into the tube to homogenise the sample. Repeat this step around 5 times before pulling as much of the liquid lysate into the syringe as possible and transferring to a clean RNase-free tube.
3. Centrifuge for 2 minutes at 10,000 – 14,000 x g.
4. Transfer the supernatant into a clean RNase-free tube.
5. Pulse vortex the lysate for 30 seconds and add 225 µL of 100 % ethanol, using the pipette to homogenise.
6. Transfer 500 µL of the lysed sample including any precipitate into the NAB Nanosep device inside a receiver tube.
7. Centrifuge for 60 seconds at 10,000 – 14,000 x g. Discard the flow-through but retain the receiver tube for the next step.

   If the sample volume >500 µL, centrifuge successive aliquots of the sample in the same NAB Nanosep device, discarding any flow through after each centrifugation but retaining the receiver tube.

   If DNA digestion is required, proceed to step 8. Otherwise proceed to step 9.

8. Optional DNA Digestion step:
   a. Add 350 µL of wash buffer 1 to the NAB Nanosep device and centrifuge for 60 seconds at 10,000 – 14,000 x g. Discard the flow-through but retain the receiver tube.
   b. Add 80 µL of prepared DNase I and supplied buffer solution (See bottle and packaging for preparation instructions). Centrifuge for a few seconds collect fluid from the sides of the tube.
   c. Pipette this prepared mixture directly onto the filter membrane in the NAB Nanosep device used in the previous steps. Incubate at room temperature for 15 minutes.
   d. Add 350 µL of wash buffer 1 and centrifuge for 60 seconds at 10,000 – 14,000 x g. Discard the flow-through, retain the receiver tube and proceed to step 10.
9. Wash the NAB Nanosep device with 500 µL of wash buffer 1 and centrifuge for 60 seconds at 10,000 – 14,000 x g. Discard the flow-through but retain the receiver tube for the next step.
10. Add 500 µL of wash buffer 2 to the NAB Nanosep device and centrifuge for 60 seconds at 10,000 – 14,000 x g. Discard the flow through but retain the receiver tube for the next step.
11. Add another 500 µL of wash buffer 2 to the NAB Nanosep device and centrifuge for 2 minutes at 10,000 – 14,000 x g. Discard the flow through and the receiver tube.
12. Transfer the NAB Nanosep device into a clean receiver tube (provided) and centrifuge again for 60 seconds at 10,000 – 14,000 x g. This will ensure no carry-over of the buffer occurs. Discard the flow through and the receiver tube.
13. Place the NAB Nanosep device insert into the final clean receiver tube (provided). Add 50 µL of RNase-free water (or elution buffer) directly onto the centre of the membrane inside the NAB Nanosep device inserts.
14. Close the lid and leave to stand at room temperature for 1 minute.
15. Centrifuge for 60 seconds at 10,000 – 14,000 x g to elute the RNA into the receiver tube.
16. Optional: Repeat the elution step with a further 50 µL RNase-free water in the same device, with the same receiver tube.

Storage of RNA

Purified RNA can be stored in RNase-free water at -20 ºC or -70 ºC for 1 year.

Quantification of RNA

RNA concentration can be determined by measuring the absorbance at 260 nm (A260) in a spectrophotometer (see details below). For small quantities of RNA however, it can be difficult to determine these amounts photometrically. Smaller quantities of DNA can be accurately quantified using fluorometric quantification.

Spectrophotometric quantification of RNA

A_{260} readings should be greater than 0.15 to ensure significance. An absorbance reading of 1.0 at 260 nm corresponds to 44 µg of RNA per mL. This is only valid for measurements at neutral pH however. As a result, if it is necessary to dilute the RNA sample, ensure that the dilution buffer is of neutral pH.

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