Thomas Scientific

PALL Laboratory

Protocol

Protocol for Total RNA Purification from Cultured Yeast Cells 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	Part Number
Pall Laboratory	Nucleic Acid Binding (NAB) Nanosep Centrifugal Device	ODNABC33, ODNABC34
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

Table 2

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

Supplier	Product Description	Part Number
Qiagen	DNaseI Set	79254 / 79256
Qiagen	Buffer RLT	79216
Qiagen	Buffer RPE	1018013
Qiagen	Buffer RDD	1011132
Qiagen	Buffer RW1	1053394
Qiagen	Nuclease-free water	129115
VWR	Zymolase	IC320921
VWR	DTT (25 g)	97063-758
VWR	D-(+)-Sorbitol	97062-202
VWR	EDTA, pH 8 (0.5 M)	BDH7830-1

2. Instruments

- Microcentrifuge
- Spectrophotometer
- Vortex

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.
- For each NAB Nanosep device insert there are three receiver tubes. This is enough to complete the below process. Use only the supplied receiver tubes with the NAB Nanosep device.
- 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. Preparations

Table 3

Solutions to be prepared prior to commencing your extraction.

Name Solution	Preparation
Buffer RLT + DTT	20 µL of DTT (2 M) per 1 mL Buffer RLT
Buffer RLT	Re-dissolve precipitate (if present)
Buffer RPE	Before first use, addition of ethanol 100 % (see volume on bottle)
DNaseI in RDD	Dissolve DNaseI in dH_2O and mix 10 µL in 70 µL of Buffer RDD - optional
Lysis buffer + Sorbitol	1 M Sorbitol + 0.1 M EDTA, pH 7.4 – 8. Before use, add 20 mM DTT + Zymolase (1-10 mg / g pellet)

5. Protocol

1. Estimate the quantity of cells before starting. Cell concentrations up to 10^7 may be used.
2. Centrifuge the culture at 1,000 x g for 5 minutes to pellet the appropriate number of cells. Remove the supernatant carefully by aspiration.
3. Resuspend the cells in 100 µL of prepared lysis Buffer containing Zymolase and incubate for 10 – 30 minutes at 30 °C, shaking gently.
4. Add 350 µL of Buffer RLT (supplemented with DTT) per tube. Vortex the mixture vigorously.
5. Add 250 µL of 100% non-denatured ethanol to the homogenised lysate. Mix well by pipetting but DO NOT centrifuge.
6. Transfer up to 500 µL of the lysed cells to the NAB Nanosep device insert inside a receiver tube, including any precipitate. Close the lid and centrifuge for 60 seconds at 10,000 – 14,000 x g. Discard the flow-through but re-use the collection tube for the next step.
7. Repeat the previous step if samples are greater than 500 µL using the same device and discarding the flow-through.

8. Optional DNase digestion steps: (If you do not require DNase digestion step move directly to step 9) (See supplier instructions for DNase I preparation)
   a. Add 350 µL of Buffer RW1 to the NAB Nanosep device insert. Close the lid and centrifuge for 60 seconds at 10,000 – 14,000 x g to wash the membrane.
   b. Discard the flow-through and retain the receiver tube for the next step.
   c. Add 80 µL of the prepared DNase I solution in Buffer RDD directly on to the NAB Nanosep device filter membrane.
   d. Incubate at room temperature for at least 15 minutes.
   e. Add 350 µL Buffer RW1 to the NAB Nanosep device. Close the lid and centrifuge at 10,000 – 14,000 x g for 60 seconds. Discard the flow-through, retain the receiver tube and proceed to step 10.
9. Wash the NAB Nanosep device membrane with 500 µL of Buffer RW1 and centrifuge for 1 minute at 10,000 – 14,000 x g. Discard the flow through and retain the receiver tube for the next step.
10. Add 500 µL of buffer RPE to the NAB Nanosep device, close the lid and centrifuge for 60 seconds at 10,000 – 14,000 x g. Ensure all the solution has passed through the filter membrane to avoid any carryover.
11. Repeat step 10 but centrifuge for 2 minutes.
12. Carefully remove the NAB Nanosep device insert, being careful not to allow the filtrate to contact the insert, and discard the receiver tube.
13. Place the NAB Nanosep device insert into a clean receiver tube (provided), close the lid and centrifuge for 60 seconds at 10,000 – 14,000 x g.
14. Discard the filtrate tube and place the NAB Nanosep device insert into a clean receiver tube.
15. Add 50 µL of RNase-free water directly on to the NAB Nanosep device insert filter membrane. Close the lid and incubate at room temperature for 1 minute. Centrifuge for 60 seconds at 10,000 – 14,000 x g to elute the RNA from the filter membrane.
16. Optional:

    Repeat the elution step with a further 50 µL RNase-free water in the same device, in the same receiver tube.

Storage of RNA

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

Quantification of RNA

RNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) 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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2/20, PDF, GN19.1210

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