Thomas Scientific

PALL Laboratory

Protocol

Standard Reagent Protocol for Total RNA Purification from Cultured Cells Using Pall AcroPrep™ Advance 96-well Long Tip Filter Plate for Nucleic Acid Binding

1. Consumables and Reagents

Table 1

Consumables for RNA purification using standard reagent protocol

Supplier	Product Description	PN
Pall Laboratory	Pall AcroPrep Advance 96-well Long Tip Filter Plate for Nucleic Acid Binding	8133
Greiner Bio-One	1 mL MASTERBLOCK◆ 96-Well Deep Well Microplates	780261
Corning Axygen◆	2.2 mL 96-Well Deep Well Plates, Square Wells	P-2ML-SQ-C
Greiner Bio-One	500 µL MASTERBLOCK 96-Well Deep Well Microplates	786201
Corning Axygen	Sealing Tape	PCR-SP-S

Table 2

Reagents for RNA purification using standard reagent protocol

Buffer	Supplier	Product Description	PN
GTC Lysis Buffer	Amresco	Guanidine Thiocyanate (GTC)	0380-500G
	Amresco	1 M Tris-HCl pH 7.5	E691-500ML
	Amresco	0.5 M EDTA	E177-500ML
DNase I	Amresco	DNase I (50,000 units)	0649-50KU
	Amresco	1 M Tris-HCl pH 7.5	E691-500ML
	Amresco	5 M NaCl	E529-500ML
	Amresco	1 M $CaCl_2$	E506-500ML
	Amresco	1 M $MgCl_2$	E525-100ML
RNA Wash Buffer	Amresco	Potassium Acetate	0698-1KG
	Amresco	1 M Tris-HCl pH 7.5	E691-500ML
	Amresco	RNase Free Water (1 L)	E476-1L
	Amresco	Ethanol, Anhydrous	E193-500ML

2. Buffer Compositions

Table 3

Buffers and their compositions for RNA purification using standard reagent protocol

Buffer	Composition
GTC Lysis Buffer	4 M GTC, 50 mM Tris-HCl (pH 7.5), 25 mM EDTA
GTC Wash Buffer (1:4 dilution of GTC lysis buffer in water)	1 M GTC, 12.5 mM Tris-HCl (pH 7.5), 6.25 mM EDTA
RNA Wash Buffer	60 mM Potassium Acetate, 10 mM Tris-HCl (pH 7.5), 60% EtOH
DNase I Buffer	40 mM Tris pH 7.5, 10 mM NaCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$

3. Instruments

- Vacuum manifold (Pall, PN: 5017)
- Vacuum/pressure pump
- Centrifuge with plate holders

4. Important Points Before Starting

- All steps are carried out at room temperature (15 – 25 °C).
- All vacuum steps take place at 50.8 kPa (15 in. Hg). Pall recommends covering the top of the Pall AcroPrep Advance 96-well Long Tip Filter Plate for Nucleic Acid Binding (Pall NAB plate) with a sheet of sealing film backing before applying vacuum. The backing will close off empty wells and prevent vacuum pressure drop, thereby facilitating the filtration process.

5. Standard Reagent Protocol

1. Transfer cultured cells (up to 4 x 10^5 cells/well) to 1 mL MASTERBLOCK 96-well deep well plate.
2. Centrifuge cell suspension at 300 x g for five minutes and remove supernatant by careful aspiration.

NOTE: It is import to remove as much of the remaining growth medium or wash buffer as possible before lysing cells.

3. Add 150 µL of GTC Lysis Buffer to each well of the MASTERBLOCK 96-well deep well plate. Seal the plate with sealing tape and vigorously shake the plate back and forth while keeping it flat on the bench.
4. Prepare the vacuum manifold. Place 2 mL MASTERBLOCK 96-well deep well plate in the vacuum manifold base and position the Pall NAB plate on top of the manifold collar.
5. Remove the sealing tape of MASTERBLOCK plate and add 70% EtOH (150 µL/well) and mix thoroughly.
6. Transfer the lysates to corresponding wells of the Pall NAB plate. Apply vacuum for one minute until all lysate has passed through the Pall NAB plate.
7. Add RNA Wash Buffer (170 µL/well) and apply vacuum to clear the buffer. Repeat this step one more time.
8. Add 80 µL DNase I (0.5 U/µL DNase I in DNase I Buffer) solution to each well and incubate for 20 minutes.
9. Add 170 µL of GTC Wash Buffer per well and apply vacuum to clear the buffer. Repeat this step one more time.

10. Add 250 µL of RNA Wash Buffer per well and vacuum or centrifuge (2 min at 1,500 x g) to clear the buffer. Repeat this step one more time.
11. Seal Pall NAB plate with adhesive tape seal and place on to of a 500 µL MASTERBLOCK plate. Transfer the stacked Pall NAB plate and collection plate to centrifuge. Centrifuge at 1,500 x g for two minutes to completely remove RNA Wash Buffer and dry the membrane.
12. Remove adhesive tape seal from the Pall NAB plate. Place Pall NAB plate on top of new 500 µL MASTERBLOCK collection plate.
13. Resuspend the RNA by adding 100 µL/well RNA-free water to each well, and incubate the plate for 1 min at room temperature. Seal the plate with sealing tape during the incubation. Perform the centrifugation at 1,500 x g for four minutes to recover the RNA.
14. The purified RNA samples can be used for downstream applications or stored at -80 °C by covering the plate tightly with a sealing tape.

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