PALL Laboratory

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Protocol

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

(Adapted from Qiagen RNeasy◆ 96 RNA purification protocol)

1. Consumables and Reagents

Table 1

Consumables for RNA purification using commercial reagent protocol

Supplier	Product Description	PN
Pall Laboratory	AcroPrep 96-well Advance Filter Nucleic Acid Binding Plates	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 commercial reagent protocol

Supplier	Product Description	PN
Qiagen	Buffer RLT (220 mL)	79216
Qiagen	Buffer RW1 (220 mL)	1053394
Qiagen	Buffer RPE (55 mL)	1018013
Amresco	RNase Free Water (1 L)	E476-1L
VWR International	DNase I (50,000 units)	0649-50KU
Qiagen	Buffer RDD (35 mL)	1011132
Amresco	Ethanol, Anhydrous	E193-500ML

2. Instruments

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

3. 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.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add four volumes of ethanol (96 – 100%) to obtain a working solution of Buffer RPE.

4. Commercial Reagent Protocol

1. Transfer appropriate number of cultured cells (up to 400 x 10^3 cells/well) to 1 mL MASTERBLOCK 96-well deep well plate.
2. Centrifuge cells at 300 x g for 5 min and completely remove supernatant by careful aspiration.
3. Add Buffer RLT (150 µL/well) and vigorously shake the plate back and forth while keeping the MASTERBLOCK plate flat on the bench for 10 s. Rotate the plate by 90° and shake the plate again for 10s.
4. Add 70% ethanol (150 µL/well), and mix it by pipetting.
5. Prepare the vacuum manifold. Place 2 mL MASTERBLOCK plate in the vacuum manifold base and position the Pall NAB plate on top of the manifold collar.
6. Transfer the sample mixture (maximum 300 µL) of each sample from step 4 to the corresponding well of the Pall NAB plate. Apply vacuum until transfer is complete. Switch off and release vacuum.
7. Add 80 µL of DNase I mix (0.5 U/µL DNase I in DNase I Buffer) directly onto the membrane of the each well of the Pall NAB plate. Seal Pall NAB plate using sealing tape.
8. Incubate the plate at room temperature for 15 min.
9. Remove sealing tape from the Pall NAB plate and add Buffer RW1 (1 mL/well) to each well of the Pall NAB plate. After five min incubation, switch on the vacuum and apply vacuum. Empty the waste in the 2 mL deep well plate by lifting the Pall NAB plate from the base. Reassemble the vacuum manifold (deep well waste collection plate can be reused).
10. Add Buffer RPE (1 mL/well) to the Pall NAB plate, and apply vacuum until transfer is complete. Switch off and release vacuum.
11. Repeat steps 9 and 10.
12. 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 10 min to completely remove Buffer RPE and dry the membrane.
13. Place the Pall NAB plate on top of new 500 µL MASTERBLOCK collection plate.
14. Resuspend the RNA by adding 70 µL/well RNA-free water to each well. Seal the plate with sealing tape and incubate for one min at room temperature. Centrifuge at 1,500 x g for four min to recover the RNA eluate in collection plate.
15. If desired, recovery of RNA can be improved by performing a second elution with another 70 µL of RNA-free water.
16. The purified RNA samples can be used for downstream applications or stored at -80 °C after covering the plate tightly with sealing tape.

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