PALL Laboratory

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Protocol

Protocol for Genomic DNA 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 gDNA purification

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
Axygen	Sealing Tape	PCR-SP-S

Table 2

Reagents for gDNA purification

Supplier	Product Description	PN
Qiagen	Buffer AL (264 mL)	19075
Qiagen	Buffer AW1 (242 mL)	19081
Qiagen	Buffer AW2 (324 mL)	19072
Amresco	Tris-EDTA Buffer, pH 8.0	E112-500ML
Amresco	Proteinase K	0706-100MG
Amresco	Ethanol, Anhydrous	E193-4L
Amresco	PBS (500 mL)	K812-500 mL

2. Instruments

- Vacuum manifold (Pall, PN: 5017)
- Vacuum/pressure pump
- Centrifuge with plate holders (maximum 1,500 x g)

3. Important Points Before Starting

- Ensure that ethanol has NOT been added to Buffer AL.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96 – 100%) as indicated on the bottle to obtain a working solution. Before each use, mix Buffer AW1 by inverting several times.
- Be sure to retain the closed-cell polyethylene foam packaging pad that comes with Pall AcroPrep Advance 96-well Long Tip Filter Plate for Nucleic Acid Binding (Pall NAB plate). It can be used to distribute pressure applied to sealing film during mixing steps and prevent its release.
- All centrifugation steps are carried out at room temperature (20 °C).
- Washing steps by vacuum filtration can be replaced by centrifugation at 1,500 x g for two minutes unless noted otherwise, ensure that Pall NAB plate is covered with sealing film during centrifugation to prevent cross-contamination between samples.
- During vacuum filtration, cover the top of the Pall NAB plate with the backing of a sealing film. The backing will close off empty wells and prevent vacuum pressure drop.
- Preheat an incubator to 56 °C for use in step 5. For best results, immediately proceed to next step upon completion of 56 °C incubation to minimize heat loss.
- Pall recommends preheating the elution buffer to 60 °C to improve yield.

4. Protocol

1. Seed appropriate number of cultured cells (up to 5 x 10^6 cells/well) in a 1 mL S block plate and centrifuge for 5 min at 300 x g. Aspirate media and resuspend the pellets in 200 µL/well PBS. Add 20 µL/well of proteinase K to each sample and continue with step 2.
2. Add 200 µL/well Buffer AL (without added ethanol) to each sample and proceed immediately to step 3.
3. Seal the plate with sealing film. Place the polyethylene foam packaging pad that comes with Pall NAB filter plates on top of the sealing tube, followed by an inverted empty 96-well plate. Clamp the stacked plates with both hands and vigorously shake up and down for 15 s.

IMPORTANT: The plate must be vigorously shaken up and down to obtain a homogeneous lysate. Genomic DNA will not be sheared by vigorous shaking and inverting the plate is not sufficient for mixing. The lysate and Buffer AL should be mixed immediately and thoroughly to yield a homogeneous solution.

4. Collect lysate at bottom of wells by a brief centrifugation of the plate. Allow centrifuge to reach 1,500 x g, and then stop the centrifugation.
5. Incubate at 56 °C for 10 min. Place a weight on top of the plate during the incubation. Mix occasionally during incubation, or place on a rocking platform.

NOTE: For best results, immediately proceed to next step upon completion of 56 °C incubation.

6. Carefully remove the sealing tape, and add 200 µL/well ethanol (96 – 100%) to each sample.
7. Seal the plate with sealing film. Place the polyethylene foam packaging pad that comes with Pall NAB plates on top of the sealing tube, followed by an inverted empty 96-well plate. Clamp the stacked plates with both hands and vigorously shake up and down for 15 s. Collect lysate at bottom of wells by a brief centrifugation of the plate. Allow centrifuge to reach 1,500 x g, and then stop the centrifugation.
8. Place 2 mL S-block in base of vacuum manifold, and place Pall NAB plate on top of manifold.
9. Remove and discard the sealing film from the plate with the lysate solution. Carefully transfer the lysis mixture (maximum 900 µL) of each sample from step 7 to the corresponding well of the Pall NAB plate on the vacuum manifold.

10. Cover top of filter plate with the backing of a sealing film and start vacuum filtration at 85 kPa (25 in. Hg) (backing will close off empty wells and prevent vacuum pressure drop).
    - Should some wells not empty, place the filter plate on 0.5 mL S-block, seal filter plate with the adhesive tape seal and centrifuge for four min at up to 1,500 x g. The adhesive tape seal prevents cross-contamination between samples during centrifugation.
    - Should lysate remain in any of the wells, centrifuge for another four minutes.
11. Remove the sealing film backing and add 500 µL/well Buffer AW1 to each sample.

NOTE: Ensure that ethanol has been added to Buffer AW1 prior to use.

12. Cover top of filter plate with the sealing film backing and start vacuum filtration at 85 kPa (25 in. Hg).
13. Remove the sealing film backing and add 500 µL/well Buffer AW2 to each sample.

NOTE: Ensure that ethanol has been added to Buffer AW2 prior to use.

14. Centrifuge uncovered plate for 15 min at up to 1,500 x g to ensure evaporation of residual ethanol which might otherwise inhibit downstream reactions.
15. Place each Pall NAB plate in the correct orientation on a new 500 µL receiver plate.
16. Resuspend DNA by adding 200 µL/well Tris-EDTA buffer (preheated to 60 °C) to each sample, and incubate for 1 min at room temperature (15 - 25 °C). Centrifuge for four minutes at up to 1,500 x g to elute the DNA.
17. Recommended: For maximum DNA yield, repeat step 15 with another 200 µL Tris-EDTA buffer.
18. The purified genomic DNA samples can be used for downstream applications or stored at 2 – 8 °C by covering the plate tightly with a sealing tape.

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