Thomas Scientific

PALL Laboratory

Protocol

Protocol for Plasmid DNA Purification from Bacterial Cells Using Pall AcroPrep™ Advance 96-Well Long Tip Filter Plate for Nucleic Acid Binding

1. Consumables and Reagents

Table 1

Consumables for plasmid DNA purification

Supplier	Product Description	PN
Pall	Pall AcroPrep Advance 96-well Long Tip Filter Plate for Nucleic Acid Binding	8133
Pall	AcroPrep Advance Filter Plate for Lysate Clearance	8175
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
VWR International	VWR Rayon Films for Biological Cultures	60941-086
Corning Axygen	Sealing Tape	PCR-SP-S

Table 2

Reagents for plasmid DNA purification

Buffer	Supplier	Product Description	PN
Resuspension	Amresco	1 M Tris, pH 8.0	E199-500 mL
	Amresco	0.5 M EDTA	E177-500 mL
	Amresco	RNase A (10 mg/mL)	E866-5 mL
Lysis	Amresco	10 M NaOH	E584-500 mL
	Amresco	20% SDS	0837-500 mL
Neutralization	Amresco	Potassium Acetate	0698-2.5 KG
Binding	Amresco	Guanidine-HCl	0287-500 G
Wash	Amresco	EtOH	E193-4 L
	Amresco	1 M Tris, pH 7.5	E691-500 mL
Elution	Amresco	10 mM Tris, pH 8.0, 1 mM EDTA	E112-500 mL

2. Buffer Compositions

Table 3

Buffers and their compositions for plasmid DNA purification

Buffer	Composition
Resuspension Buffer	50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 µg/mL RNase A
Lysis Buffer	200 mM NaOH, 1% SDS
Neutralization Buffer	3.0 M Potassium Acetate, pH 5.5
Binding Buffer	6 M Guanidine-HCl
Wash Buffer	10 mM Tris, pH 7.5, 80% Ethanol
Elution Buffer	10 M Tris-HCl, pH 8.0, 1 mM EDTA

3. Instruments

- Vacuum manifold (Pall, PN: 5017)
- Vacuum/pressure pump
- Centrifuge with plate holders (Maximum 5,000 x g)
- Micro plate shaker

4. Important Points Before Starting

- All steps are carried out at room temperature (20 – 25 °C), except where noted
- Pall recommends retaining the polyethylene foam packaging pad that comes with Pall AcroPrep Advance 96-well Long Tip Filter Plate for Nucleic Acid Binding (Pall NAB plates) as it can help prevent sealing film release during mixing steps. Before mixing, simply place the foam pad on top of the sealed plate, followed by an inverted empty 96-well plate. Clamp the plate stack with both hands and mixing by inversion can be accomplished without the risk of sealing film release.
- For yield determinations, the elution volume in the receiver plate can be determined by weighing the receiver plate before and after elution and dividing the weight difference by the number of sample containing wells.

5. Protocol

1. Inoculate from single bacterial colonies each well of a 96-well deep well plate filled with 1.5 mL LB broth supplemented with 100 µg/mL ampicillin.
2. Seal the plate with gas permeable sealing film (VWR Rayon Films for biological cultures) and incubate with vigorous shaking for 20 – 24 h at 37 °C (with non-permeable tape, aeration can be achieved by piercing 2 – 3 holes/well in the tape with a needle).
3. Harvest the bacterial cells in the deep well plate by centrifugation for 10 min at 2,100 x g preferably with centrifuge chamber at 4 – 10 °C. The deep well plate should be covered with sealing tape during centrifugation.
4. Remove sealing film and decant supernatant over a waste container. Tap the inverted block firmly on a paper towel to remove any remaining droplets of medium.
5. Add Resuspension Buffer (150 µL/well). Seal plate with sealing tape and resuspend the pellets by vortexing.

6. Add Lysis Buffer (150 µL/well). Seal the plate sealing tape and mix by inverting the plate 4 – 6 times. We recommend the following to prevent sealing tape release: Place the foam packaging pad that comes with Pall NAB plates on top of the sealing tape, followed by an inverted empty 96-well plate. Clamp the stacked plates with both hands and mix by inversion.
7. Remove tape and add Neutralization Buffer (150 µL/well). Dry top of plate and mix by inversion as in step 6.
8. Lysate clarification can be carried out either by vacuum filtration or by centrifugation:
   a. By vacuum filtration
      i. Place Pall NAB plate on top of 350 µL collection plate and place into base of vacuum manifold.
      ii. Place the lysate clarification plate on top of vacuum apparatus.
      iii. Transfer cell lysates to corresponding wells of lysate clearance plate and apply vacuum at 34 kPa (10 in. Hg) for 1 min until all filtrate has passed through to the Pall NAB plate.
   b. By centrifugation
      i. Centrifugate the 2 mL deep well plate with lysate solution for 5 min at 1,500 x g to pellet precipitate.
      ii. Transfer supernatant to Pall NAB plate.
9. Place 2 mL waste collection plate in bottom of vacuum apparatus and move the Pall NAB plate to the top of the manifold.
10. Add Binding Buffer (450 µL/well) and pipette up and down to mix.
11. Apply vacuum at 51 kPa (15 in. Hg) for slow vacuum and discard the filtrate.
12. Add Wash Buffer (600 µL/well).
13. Apply vacuum, then discard the filtrate. Discarding is unnecessary if using 2 mL collection plate or filtering directly to waste.
14. Repeat steps 12 and 13.
15. Place the plate on collection plate and centrifuge at 1,500 x g for 5 min to ensure removal of residual ethanol. If needed, blot outlet of filter plate to ensure removal of ethanol droplets upon completion of centrifugation.
16. Add Elution Buffer (70 µL/well) and incubate the plate at room temperature for one minute.
17. Purified plasmid DNA can be eluted either by vacuum filtration or by centrifugation.
    a. By vacuum filtration
       i. Place clean collection plate into vacuum manifold.
       ii. Place filter plate on top of vacuum manifold, apply vacuum at 50.8 kPa (15 in. Hg) for 1 min until all elution buffer has passed through the DNA binding plate.
    b. By centrifugation
       i. Place purification filter plate on top of clean collection plate and centrifuge at 1,500 x g for 5 min.
18. The purified plasmid DNA samples can be used for downstream applications or stored at -20 °C or -70 °C by covering the plate tightly with sealing tape.

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