Protocol

Protocol for Plasmid DNA Purification from Bacterial Cells without Lysate Clearance using Pall AcroPrep™ Advance 96-Well Long Tip Filter Plates for Nucleic Acid Binding

1. Consumables & Reagents

Table 1

Consumables for plasmid DNA purification

Supplier	Product Description	PN
Pall	AcroPrep Advance 96-Well Long Tip Filter Plate for Nucleic Acid Binding	8133
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	E199-500ML
	Amresco	0.5M EDTA	E177-500ML
	Amresco	RNase A (10 mg/mL)	E866-5ML
Lysis	Amresco	10 M NaOH	E584-500ML
	Amresco	20% SDS	0837-500ML
Neutralization	Amresco	Ammonium Acetate	0103-2.5KG
Wash	Amresco	EtOH	E193-4L
	Amresco	1 M Tris, pH 7.5	E691-500ML
Elution	Amresco	10 mM Tris, pH 8.0, 1 mM EDTA	E112-500ML

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 Ammonium Acetate, pH 5.5
Wash Buffer	10 mM Tris, pH 7.5, 80% Ethanol
Elution Buffer	10 mM 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 × *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 Plates for Nucleic Acid Binding (Pall NAB plates) as it can help prevent sealing tape 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 tape 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 2.2 mL 96-well deep well plate filled with 1.25 mL/well 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 × *g* in a centrifuge with a rotor for a 96-well adapter, preferably with centrifuge chamber at 4 – 10 °C. The deep well plate should be covered with sealing tape during centrifugation.
4. While bacterial cells are pelleted, prepare Pall NAB Plate for use by adding 100 µL Elution Buffer to each well. Cover unused wells of Pall NAB Plate with sealing tape. Place 2 mL waste collection plate in base of vacuum manifold and place Pall NAB plate on top of manifold. Apply vacuum until buffer has passed through. Leave the plate until ready for use in step 10.
5. Upon completion of centrifugation step, remove sealing tape from deep well plate and decant supernatant over a waste container. Tap the inverted deep well plate firmly on a paper towel to remove any remaining droplets of medium.

6. Add Resuspension Buffer (150 µL/well). Seal plate with sealing tape and resuspend the pellets by vortexing until no cell clumps are visible.
7. Add Lysis Buffer (150 µL/well). Seal the plate sealing tape and mix by inverting the plate 6 times. We recommend the following to prevent sealing tape release: Place the foam packaging pad that comes with Pall NAB filter 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.
8. Remove tape and add Neutralization Buffer (150 µL/well). Dry top of plate, seal the plate and mix by inversion as in step 7.
9. Remove tape and add isopropanol (300 µL/well). Dry top of plate, seal the plate and mix by inverting the plate 2 times as in step 7.
10. Transfer the lysate to Pall NAB plate on vacuum manifold (prepared for use in step 4). Apply vacuum at 51 kPa (15 in. Hg) for slow vacuum. Discard the filtrate from waste collection plate. The waste collection plate can be reused.
11. Add Wash Buffer (750 µL/well). Apply vacuum until Wash Buffer has passed through all wells. Switch off vacuum and discard the filtrate from waste collection plate.
12. Repeat step 11.
13. Place the Pall NAB plate on a collection plate and centrifuge at 1,500 × *g* for 5 min to ensure removal of residual ethanol from wash buffer. If needed, blot outlets of filter plate on absorbent paper to ensure removal of wash buffer droplets upon completion of centrifugation.
14. Add Elution Buffer (75 µL/well) and incubate the plate at room temperature for one minute.
15. 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 × *g* for 5 min.
16. Use new sealing tape to cover the collection plate containing the eluates. The purified plasmid DNA samples are ready for use in downstream applications or can be stored frozen at -20 °C or -70 °C.

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