

# A New Chromatographic Technique for Commercial Purification of Plasmid DNA and the Simultaneous Removal of Endotoxin

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## Summary

Commercial plasmid DNA purification has been reliant on chromatographic techniques such as ion exchange chromatography and gel filtration. Any new process for the isolation of plasmid DNA for gene therapy applications must produce DNA of high purity, free of host cell contaminants that tend to co-purify.

Perfluorosorb S is a new, specifically engineered adsorbent for purification of supercoiled plasmid DNA by reverse phase chromatography. It consists of spherical polymeric beads with large surface areas, high porosities and a hydrophobic surface chemistry. It has a high capacity for DNA. **Capacities** up to 2mg DNA per gram of resin **have** been achieved.

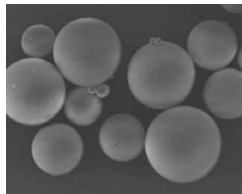
The adsorbent can be used for primary capture and purification of plasmid DNA following cell lysis and clarification. Clarified lysate from 200 ml of overnight culture inoculated with a medium copy plasmid gave a total yield of 2.7 mg supercoiled DNA.

Perfluorosorb S can also be used for endotoxin clearance and as a secondary polishing step when used in conjunction with alternative capture strategies, for example anion exchange chromatography. Endotoxin loads of over 1,000 EU/mg (LAL) have been reduced to **as low as 1.0 EU/mg (LAL)** during the polishing step. It has also been demonstrated that Perfluorosorb S can be used directly after an ion-exchange in the presence of residual salt. Load concentrations of salt at 0.175, 0.35 and 0.7 M do not affect the resin's performance.

The transfection efficiency of plasmid DNA captured directly on Perfluorosorb S is comparable to the industry standard. CCL39 Fibroblasts were transiently transfected with a green fluorescent protein containing plasmid. Transfection efficiencies of more than 60% were achieved for plasmids isolated using both Perfluorosorb S and an 'industry standard' product.

This new, highly stable adsorbent is re-useable and can be subjected to rigorous clean-in-place procedures. Perfluorosorb S can be used both for purification directly from clarified lysate (primary capture step) and in a secondary polishing step, following ion exchange primary capture, even in the presence of residual salt. These procedures also provide effective clearance of endotoxins from the purified pDNA.

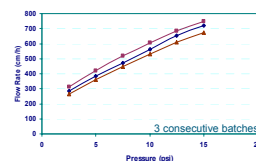
## Perfluorosorb® S for chromatography of pDNA



- New synthetic, fluorinated polymeric entity
- Spherical, porous particles
- 40 - 45mm mean particle size
- 1g ≈ 3.5 mL (packed volume)
- Pure pDNA binding capacity ~ 1.4 -1.7 mg/g
- Flow rates up to 700 cm/hr at 15 psi
- Tolerates **2000** psi in HPLC columns
- Stable in pH range 1 – 14
- Stable in 1M HCl
- Resistant to 5M NaOH
- Suited to repeat-use bioprocess applications
- Store at 2-30°C in 24% EtOH

Unique adsorbent  
Specifically engineered for pDNA  
RPC, strong HIC  
Designed for chromatography

### Pressure-flow characteristics

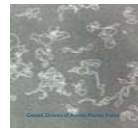


Tests performed in water  
(After packing in 70% EtOH)  
Column: 3.2 cm Ø x 15 ± 1cm  
(ca. 120 mL)

## Procedure for pDNA purification on Perfluorosorb S

Capture or polishing step

1	<b>Equilibrate column Buffer C1</b>	Perfluorosorb S supplied as a slurry in 24% (v/v) EtOH								
2	<b>Load sample</b>	Decant supernatant, replace with 70% (v/v) EtOH								
3	<b>Wash column Buffer C2</b>	Pack and run column (>2CV) in 70% EtOH at 70 – 100 cm/hr								
4	<b>Elute pDNA Buffer C3</b>	Buffer C1: Equilibrate, load; Buffer C2: Wash; Buffer C3, Elute								
5	<b>Precipitate or buffer exchange</b>	<table border="0"> <tr> <td><b>Capture</b></td> <td><b>Polishing</b></td> </tr> <tr> <td>C1: Triethylamine/Phosphate, pH 7</td> <td>C1: Triethylamine/Tris, pH 7.5</td> </tr> <tr> <td>C2: Tris-EDTA, pH 8.0</td> <td>C2: Tris-EDTA, pH 8.0</td> </tr> <tr> <td>C3: Na acetate/EtOH (8%), pH 8.5</td> <td>C3: Na acetate/EtOH (25%), pH 8.5</td> </tr> </table> <p>Precipitate pDNA with 0.7 volumes of ice-cold iso-propanol. Re-suspend pellet in buffer C1. Use 60 – 100 mL /litre of original fermentation volume, or, follow procedures for polishing</p> <p>Precipitate pDNA and re-suspend, or dilute with conc. C1, or dialyze/Dialyse against C1. Salt concentration should be below 0.5 M</p>	<b>Capture</b>	<b>Polishing</b>	C1: Triethylamine/Phosphate, pH 7	C1: Triethylamine/Tris, pH 7.5	C2: Tris-EDTA, pH 8.0	C2: Tris-EDTA, pH 8.0	C3: Na acetate/EtOH (8%), pH 8.5	C3: Na acetate/EtOH (25%), pH 8.5
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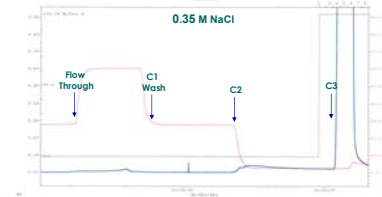
### Column operation

Equilibrate column in 5 – 10 CV C1 at 70 – 100 cm/hr  
Load in C1 at 30 – 70 cm/hr  
Wash with ~ 5CV C2 at 70 – 100 cm/hr  
Elute ~ 4CV C3 at 30 – 70 cm/hr  
Pool C3 peak  
CIP and re-equilibrate column

### Clean-in-place procedure

Flush with 2 CV de-ionised water  
Flush with 2 CV NaAc/8% EtOH  
Flush with 2CV 1M HCl, hold for 1 hour  
Flush with de-ionised water to >pH 5  
Flush with 2CV 1M NaOH, hold for 1 hour  
Wash with de-ionised water to <pH 9  
Wash with 2CV 70% EtOH  
Store or re-equilibrate in C1

## Polishing step (after anion exchange)

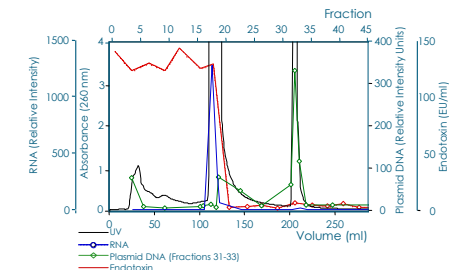


### C3 eluted DNA

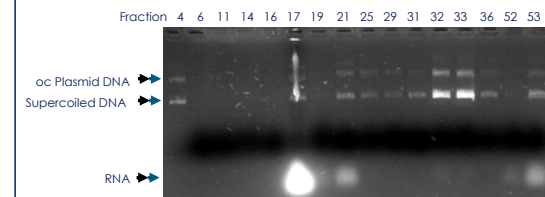
[NaCl]	% Recovered	260/280	Endotoxin (EU/mg)
0.175 M	88%	1.96	3.1
0.350 M	96%	1.89	2.9
0.70 M	99%	1.91	3.4

## Purification of pDNA from lysate

Pellet from 200 mL culture suspended in 30 mL C1  
Column: 2.5 cm Ø x 6 cm, V<sub>t</sub> = 29.5 mL  
30 mL sample (1 CV) loaded at 24.5 cm/hr (Endotoxin in flow-through)  
Column washed (RNA elution) with 3 CV C2  
pDNA eluted with 3CV C3 containing 8% EtOH  
Residual nucleic acids removed with C3 containing 25% EtOH

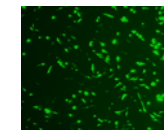


### Agarose (0.8%) electrophoresis of fractions

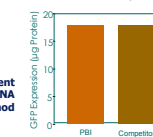
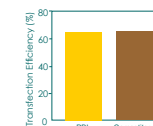


- Yield = **2.7 mg supercoiled plasmid DNA**
- Purity (260/280) = **1.96**
- Total protein = **22µg protein/mg plasmid (BCA assay)**
- < **2% total RNA by 0.8% AGE densitometry**
- Endotoxin level < **50 EU/mg DNA (BioWhittaker LAL Kinetic assay)**

## Bioavailability



Fluorescent photomicrograph of CCL39 fibroblasts transiently transfected with DNA Green Fluorescent Protein containing plasmid.



Expression level of Green Fluorescent Protein in cells transfected with DNA prepared by the indicated method

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