

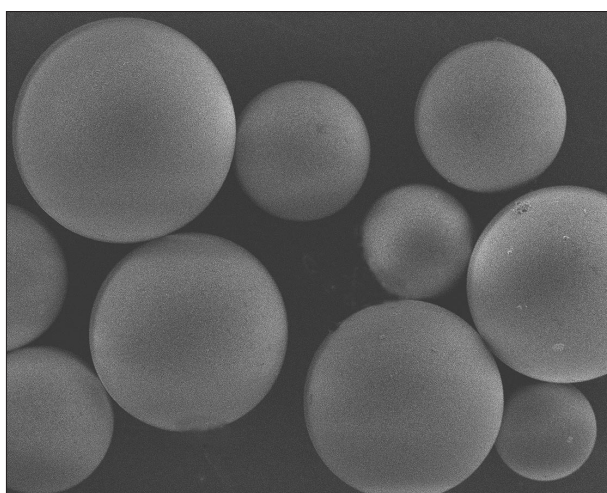
# Purification of Plasmid DNA by polishing and Endotoxin removal

**Increasing requirements for higher purity plasmid DNA preparations have driven the development of new chromatography technologies. Victor Bornsztejn reports.**

**P**erfluorosorb S is a highly fluorinated, synthetic adsorbent designed specifically for the purification of plasmid DNA from alkaline lysates of *E. coli*. Perfluorosorb is used as a reverse-phase adsorbent with ion-pairing agents in a polishing or capture mode and is suitable for both research purposes, and cGMP (therapeutic) manufacturing. Perfluorosorb is capable of resolving supercoiled plasmid DNA in high salt fractions from ion exchange and is effective in reducing endotoxin levels to below required levels.

Perfluorosorb S is an engineered, polymeric adsorbent for the purification of plasmid DNA by reverse phase chromatography. It has a highly porous structure with a high capacity for supercoiled pDNA, and is extremely robust, allowing it to be re-used after cleaning procedures are employed. It can be used for primary capture and purification following cell lysis and clarification. It can also be used for endotoxin clearance or as a polishing step when used in conjunction with alternative capture strategies, for example ion exchange chromatography.

Plasmid DNA (pDNA) and other forms of DNA bind to Perfluorosorb S in neutral buffers containing ion pairing agents such as triethylamine acetate (TEAA) or tetrabutyl ammonium phosphate (TBAP).



**Fig. 1.** Perfluorosorb S – highly fluorinated, synthetic adsorbent engineered specifically for the purification of plasmid DNA.

Endotoxins are eluted in the flow-through and residual amounts cleared together with RNA in a wash step using a Tris-EDTA buffer. Genomic DNA and non-constrained or nicked isomers of the plasmid are eluted in the wash. Plasmid DNA is desorbed from the Perfluorosorb using a high pH sodium acetate buffer containing up to 25 per cent ethanol. Ethanol concentrations and buffer compositions vary depending on whether the Perfluorosorb S is being used in a capture, or polishing mode. Preparative separations can be performed in columns up to 1 litre volume with single cycle capacities of up to 0.5 grammes pDNA. Following purification, the pDNA can be concentrated by alcohol precipitation or by ultrafiltration.

Ion pairing agents are used to enhance separations and resolution in reverse phase chromatography. The mobile phase composition is a critical variable since the solid phase is fixed. Addition of ion polar species that bind either anions or cations can greatly affect the binding between the extremely hydrophobic surface and exposed polar surface of the separation target. TBAP and TEAA are commonly used as cationic agents. TBAP, like other quaternary alkyl ammonium salts, is one of the more effective ion pairing agents promoting the retention and adsorption of highly hydrophilic species. Both TBAP and TEAA can be used with Perfluorosorb.

## Plasmid DNA and Endotoxins

Endotoxin is a lipopolysaccharide component of the cell wall of gram-negative bacteria such as *E. coli*. Endotoxins, like DNA, are negatively charged, and are therefore often co-purified with plasmid DNA when using traditional methods such as anion exchange chromatography. In *in-vivo* applications such as gene therapy and administration of DNA vaccines, the presence of endotoxin can cause fever and endotoxic shock syndrome. In research applications, endotoxin can significantly reduce transfection efficiencies in certain sensitive cell lines, and can additionally affect other applications including PCR, sequencing, cloning and restriction endonuclease digestion. Therefore the ability to effectively reduce the level of endotoxin in plasmid DNA preparations, either on the research or large manufacturing scale, is essential.

Buffer Preparations: \* Buffer C1: Triethylamine acetate (TEAA) 1M (pH 7.0) 200 ml; Tris/HCl 1 M (pH 7.5) 200 ml; adjust volume to 1000 ml with

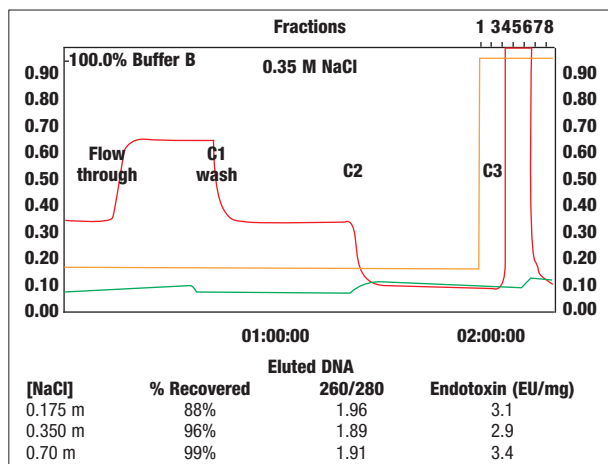


Fig. 2. Clean up of DNA after ion-exchange step.

dH<sub>2</sub>O, pH 7.5; Buffer C2: 10 x STE (0.2M Tris-HCl, 0.01M EDTA, 0.05M NaCl, pH 8.0) 100 ml; 100 per cent ethanol 20 ml; adjust volume to 1000 ml with dH<sub>2</sub>O to 1000 ml; pH 8.0; Buffer C3: Sodium acetate anhydrous salt (FW = 82.03 g) 8.2 g; 100 per cent ethanol 250 ml; adjust volume to 1000 ml with dH<sub>2</sub>O; pH 8.5.

Column Preparation: Perfluorosorb S is packed into a column following the procedure. The column is then equilibrated with Buffer C1.

- ◆ Sample Preparation: The sample solution containing plasmid DNA should be in the equilibration Buffer C1. This can be achieved in several ways, including precipitation, dialysis or diafiltration.
- ◆ Column Operation: Load the prepared sample onto the equilibrated column. After sample loading, wash the column with 2–3 column volumes of Buffer C1 to remove unbound impurities. The column is then washed with 5 column volumes of Buffer C2 to remove loosely bound impurities. Finally, the bound DNA is eluted with Buffer C3 (4 column volumes) and the eluted peak pooled.

## Results

The figure shows the results of a polishing/endotoxin removal experiment run at an initial salt concentration of 0.35M NaCl. Conductivity in mS (red line) is seen to drop with each buffer change. Supercoiled pDNA was recovered at a purity of 1.89 (A<sub>260</sub>/A<sub>280</sub>) and endotoxin was reduced to below 3 EU/mg plasmid as assessed by LAL assay. Recovery was 96 per cent. Recovery increases slightly with 0.7 M NaCl in the load and is lower at 0.175 M NaCl. Endotoxin and purity remain essentially constant.

Victor Bornsztejn is with ProMetic BioSciences, based in New Jersey, USA. [www.prometic.com](http://www.prometic.com)

This article first appeared in eLab June 2004

# Designer Genes



## Customize your Plasmid DNA Process

Life science researchers are challenged every day to find cures for fatal diseases like cancer and AIDS. Finding solutions to these genomic challenges requires innovative approaches to science.

At ProMetic Biosciences, we've developed our own innovative approach to Plasmid DNA purification to increase purity and yield.

To find out how our Perfluorosorb S highly fluorinated adsorbent can help you in your search to design the next custom genome strand, contact us today.

For more information, log on to [www.DNA.discoverprometic.com](http://www.DNA.discoverprometic.com)

Call +1.973.812.9880 or e-mail: [sales@prometic.com](mailto:sales@prometic.com)



More of what you want,  
less of what you don't.