Proteus NoEndo™

A new way to remove endotoxin from biological samples

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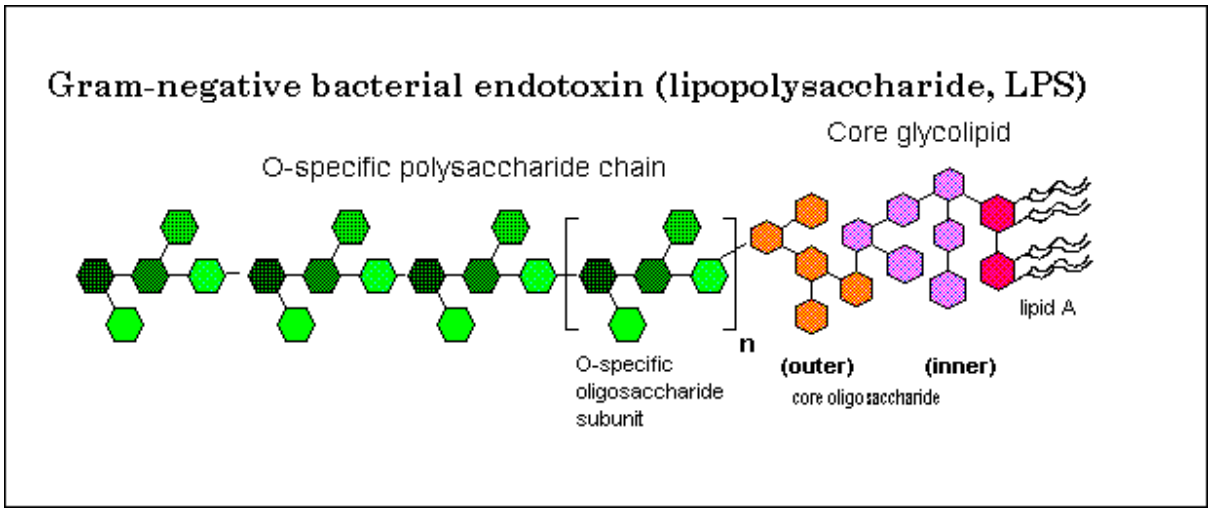
Background:

Endotoxin removal from antibodies and proteins has historically been difficult with inconsistent results. Many commercially available protocols are unable to remove endotoxin effectively and are based on non-affinity chromatography methods such as ion exchange chromatography or phase separation using Triton X-114. It is well documented (Magalhaes, P. *et al*, 2007) that there is dissatisfaction with many known commercial methods to remove endotoxin from biological products. Typically, the target protein binds almost as efficiently to the matrix as the endotoxin and cannot be recovered easily, or both endotoxin and protein elute in the flow-through fractions.

Figure 1: Schematic diagram of gram-negative bacterial endotoxin (lipopolysaccharide).



Endotoxins (Fig. 1) are the predominant lipids found in the outer membrane of gram-negative bacteria. Sub-nanogram levels of endotoxin can trigger immune responses and alter the function of many different cell types leading to problems such as fatal septic shock. Endotoxin concentrations as low as 0.3-0.4 EU/ml can induce pyrogenic shock in mammals. The removal of endotoxin is one of the most difficult downstream processes during protein or antibody purification, where laboratory water and glassware often contaminate samples. Gram-negative bacteria are widely used as vectors for the manufacture of recombinant peptides and proteins. If the final product is not certified to be free of endotoxin, many experiments may fail. Efficient and cost effective removal of endotoxin from R & D preparations is extremely challenging. Endotoxin removal for research into animal studies, transplantation, gene therapy, stem cell technologies, cell sorting and other mammalian cell treatments is vital.

Proteus NoEndo™ Spin Column Kits combine the quality separation of gravity flow columns with the speed and ease-of-use of centrifugal spin columns. The proprietary FlowGo™ technology (Fig. 2) regulates the flow rate of the sample through the high quality affinity resin matrix, increasing endotoxin removal without compromising protein recovery during centrifugation. Uniquely, the flow rate is controlled for optimal endotoxin removal in a centrifuge. The resin contains a specific affinity ligand attached to near mono-disperse 6% cross-linked agarose beads.

Figure 2: Proteus FlowGo™

Specifications:

Table 1: Proteus NoEndo™ S and NoEndo™ HC Specifications

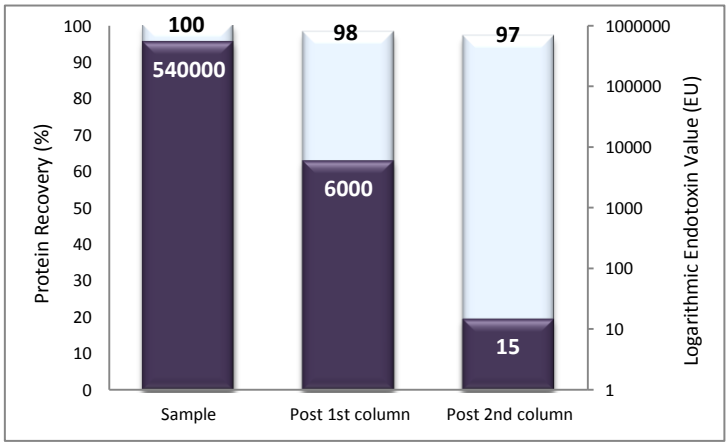
Kit	S Column	HC Column
Typical *in situ* binding capacity	30,000 EU	500,000-1,000,000 EU
Typical binding capacity per ml resin	30,000 EU/ml	450,000 EU/ml
Typical endotoxin removal after 1 pass	2-3 log reduction	2-3 log reduction
Typical endotoxin removal after 2 passes	4 log reduction	4-5 log reduction
Maximum sample load volume per pass	20 ml	20 ml
Minimum endotoxin level tested post-spin	<0.05 EU/ml	<0.05 EU/ml
Bed volume	1 ml	1.7 ml
Resin matrix	Cross-linked 6% agarose beads	Cross-linked 6% agarose beads
Bead size range	100 µm	100 µm
Recommended working pH	4-8	4-8
Color coded end-caps	Light green	Dark green

The Proteus NoEndo™ High Capacity (HC) spin column for endotoxin loads as high as 450,000 EU/ml and the Proteus NoEndo™ Standard (S) spin column for endotoxin loads of about 30,000 EU/ml.

Results:

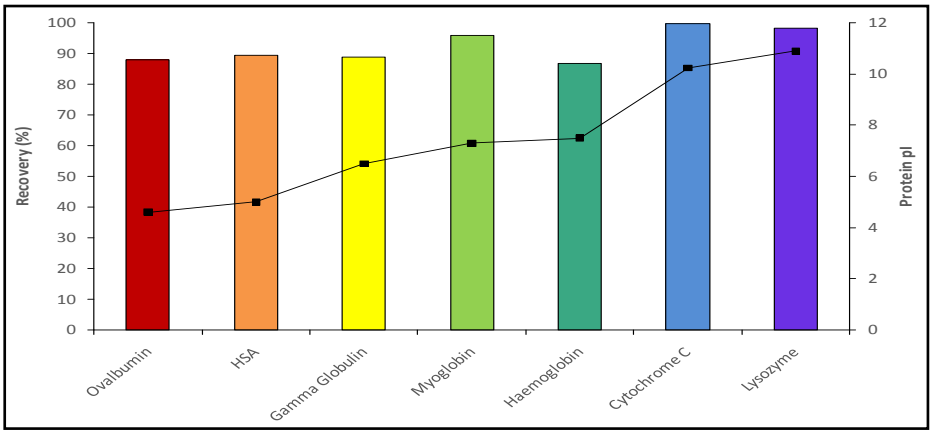
The Proteus NoEndo™ HC spin columns were pre-equilibrated with PBS (pH 7.5) and 20 ml protein (1 mg/ml BSA and 1 mg/ml IgG) samples spiked with control standard endotoxin (Charles River Laboratories, Wilmington, MA, USA). The columns were loaded and centrifuged at 100 g for 30 minutes. The filtrates were then loaded on to second columns and centrifuged at 100 g for a further 30 minutes. Endotoxin data was generated using the kinetic chromogenic LAL assay (Charles River Endosafe® plate reader). The resulting protein samples showed a 98.9% (about 2 log) removal after the first column step and a 99.997% (over 4 log) reduction in endotoxin cumulative for both filtration steps. (Fig. 3)

Figure 3: Endotoxin removal and protein recovery from 1 mg/ml BSA sample in PBS buffer.



Proteus NoEndo™ spin columns also exhibit low protein binding and a wide range of proteins can be processed regardless of their iso-electric point. Figure 4 shows that >85% recovery is achieved for various model proteins spanning the pI spectrum.

Figure 4: Protein recoveries for a wide range of proteins with different iso-electric points.



Recombinant antibody fragments are the smallest binding units of antibodies (~13 kDa). Two different recombinant antibody fragments produced in *E. coli* (post initial capture step) were loaded on to equilibrated Proteus NoEndo™ columns. Results presented in the table below show high protein recovery and significant endotoxin removal (Table 2).

Table 2: Protein recovery and endotoxin removal from two recombinant antibody fragments.

Antibody Fragments	pI	Load [Protein] mg/mL	Load EU/mg protein	Protein recovery (%)	EU/mg protein in non-bound	Endotoxin Removal
Ab Fragment 1	7.3	0.70	7,200	85	110	1.8 log
Ab Fragment 2	4.6	0.34	22,000	94	440	1.7 log

Conclusion:

The Proteus NoEndo™ spin columns contain a synthetic affinity ligand adsorbent that removes endotoxin from a wide range of proteins across the pI spectrum with recoveries of >85% and can operate in a range of conditions from acidic to neutral pH.

The columns have a high capacity for endotoxin and bind up to 450,000 EU/mL resin with low protein binding (>85% recoveries). These columns gave an endotoxin removal of about 2 log from purified recombinant antibody fragments expressed in *E. coli.* Protein recoveries can be improved by increasing the target protein concentration without detrimental impact on endotoxin binding making the Proteus NoEndo™ spin columns ideally suited for final polishing applications.

To address the issues associated with the current available endotoxin removal methods, the Proteus NoEndo™ column is an effective tool for selective removal of endotoxins. The combination of the unique FlowGo™ technology with the ease-of-use of a spin column allows rapid endotoxin removal results with high protein recovery.

References:

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