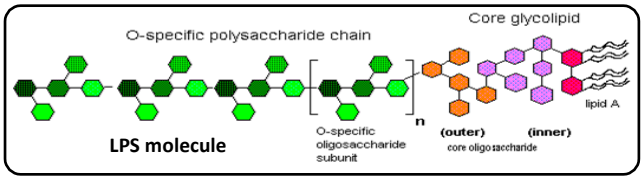
Proteus NoEndo™ M (Mini), NoEndo™ S (Standard) and NoEndo™ HC (High Capacity) Spin Column Kits: The Gold Standard

Residual endotoxin contamination in advanced biotherapy products is an expensive and often difficult contaminant to control.

Many commercially-available protocols are unable to remove endotoxins effectively and are based on non-affinity chromatography methods e.g. Ion exchange chromatography, phase separation using Triton X-114 or require time consuming and expensive affinity steps. These costly resins are often supplied as loose resin or packed in slow gravity columns.

The Proteus NoEndo™ spin column kits offer a standardised method for high grade clearance of endotoxin from recombinant proteins, antibodies and viral vectors. These agents are increasingly being designed for therapeutic applications, hence moving them forward efficiently through *in vivo* studies requires pure preparations of the samples.



Endotoxin-free preparation in less than 1 hour

The yields of a gravity flow with the speed of a spin column

Next generation Proteus kits combine the quality separation you expect from gravity flow columns with the speed and ease-of-use of spin columns. Both column formats reveal a high degree of innovation! The Standard and High Capacity columns incorporate pre-packed resin cartridges utilizing our FlowGo™ technology. The Mini columns are empty columns that incorporate our proprietary SelfSeal™ membrane technology. This ensures that there is no passage of the sample through the membrane during the batch incubation at 4^0C or at room temperature.

NoEndo S and NoEndo HC Columns:

The proprietary FlowGo™ technology regulates sample movement through the technologically-advanced affinity resin cartridge, increasing both endotoxin removal and protein recovery. Uniquely, we offer flow rate control for endotoxin removal in a centrifuge.

NoEndo M Columns:

The NoEndo™ Mini columns incorporate a SelfSeal™ membrane technology which retains the NoEndo™ resin and sample in the batch incubation chamber. When the column spun in a low speed centrifuge, the pores of the membrane dilate and the filtered eluate is collected in the bottom of the centrifuge tube.

We offer three versions of the Proteus kits: *Mini, Standard and High Capacity*

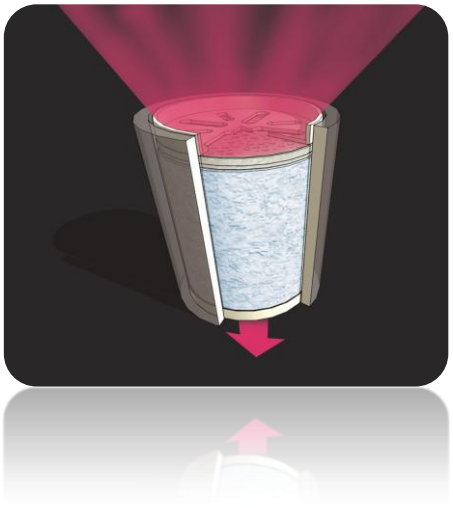
- **Proteus NoEndo™ Mini:** for endotoxin loads less than 3,000 EU
- **Proteus NoEndo™ Standard:** for endotoxin loads less than 30,000 EU
- **Proteus NoEndo™ High Capacity**: for endotoxin loads less than 1,000,000 EU

	NoEndo™ M	NoEndo™ S	NoEndo™ HC
E.coli Expressions			✓
Mammalian Expressions	✓	✓	
Insect Expressions	✓	✓	
Yeast Expressions	✓	✓	
Antibody Samples	✓	✓	
Final Polishing Steps	✓	✓	✓

Proteus NoEndo™ M, NoEndo™ S and NoEndo™ HC applications

Convenient	**For NoEndo™ S and NoEndo™ HC columns only incorporating the FlowGo™ Technology:** Pre-packed chromatography resin plug – no mess, no filling columns, no pumps, no lengthy steps and minimal optimisation required. **For NoEndo™ M columns, incorporating the SelfSeal™ Technology:** Minimal manual intervention, high capture efficiency, no dilution of sample, perfect for final polishing steps.
Easy-to-use	Full technical and application handbook including unambiguous protocols supplied with every kit.
Rapid	Endotoxin removal and high protein recovery (typically >90%) in 30 min for NoEndo™ S and NoEndo™ HC columns. For low endotoxin loads, use NoEndo Mini columns. Typically, <99.9% endotoxin losses in a single 2-3 hour incubation!
Flexible	Single use, 50 ml format columns which are simple to use. The unique spin column format permits multiple and parallel processing for high throughput applications such as process optimisation, rapid scouting and screening.
Cost-effective	Disposable columns fit in a swing bucket rotor – no expensive equipment necessary.

The FlowGo™ Advantage (NoEndo™ S and NoEndo™ HC)

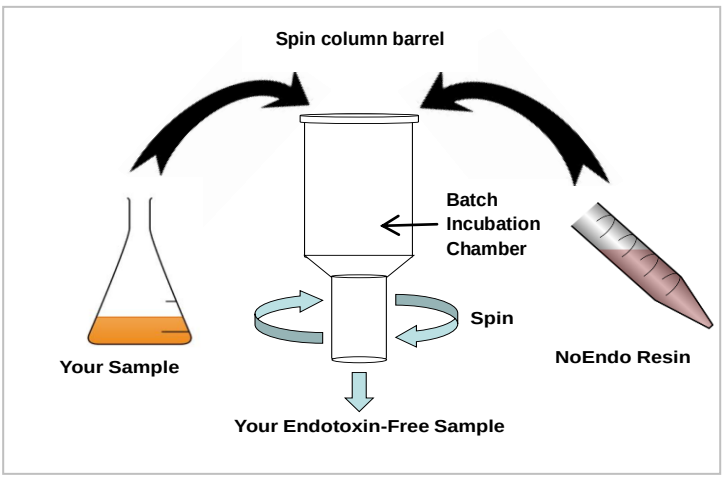


FlowGo™ is a unique technology using back pressure to enable a steady and controlled flow of sample and buffer through the affinity resin column during centrifugation. This powerful flow regulator leads to selective endotoxin capture, without compromising protein recovery and improves purification results in comparison with similar gravity flow and LC systems. The columns are supplied pre-packed and ready-to-use.

The SelfSeal™ Advantage (NoEndo™ M)

The NoEndo™ M spin columns incorporate our proprietary and NASA-inspired SelfSeal™ membrane technology. The coated membrane is specially formulated to prevent any sample from leaking into the collection tube on an orbital mixer. Batch incubation can be performed at 4^0C and at room temperature. In a centrifuge, the membrane pores dilate and the eluate, free of endotoxin, passes into the collection tube. The contact time is maximized to ensure maximum endotoxin depletion without losses of the target protein, antibody or domain antibody. Uniquely, there is also no dilution of the sample.

Proteus NoEndo™ M Workflow:



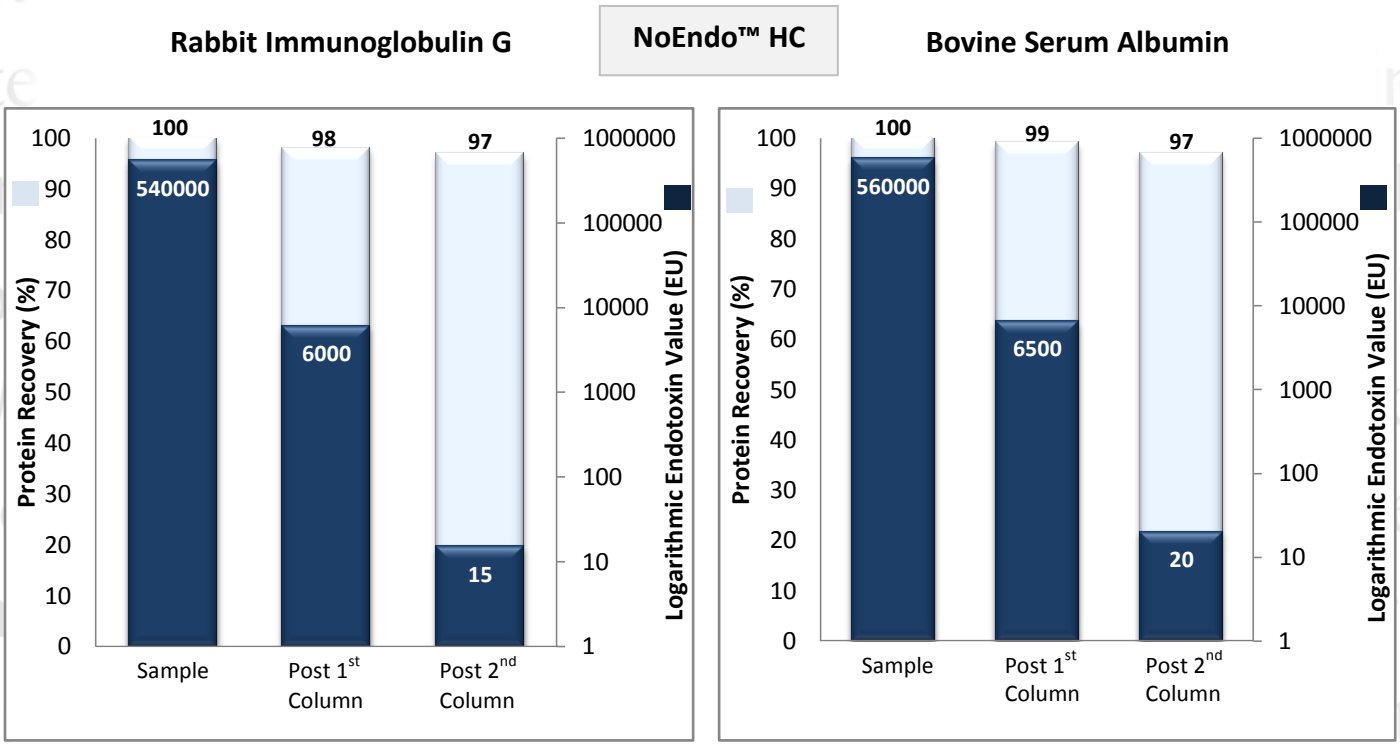


Figure 1: TheProteus NoEndo™ HC spin columns effectively remove endotoxin from BSA and rabbit IgG samples (1mg/ml) spiked with *E.coli* lysate. The Proteus NoEndo™ HC spin columns were pre-equilibrated with PBS (pH 7.5) and 20 ml protein samples were loaded and centrifuged at 100 g for 30 min. The flow throughs were loaded on to second columns and centrifuged using the same conditions. Endotoxin data was generated using the kinetic chromogenic LAL assay (Charles River Endosafe plate reader). Typically, a 4 log reduction in endotoxin was observed. The protein recoveries were determined separately with the Proteus NoEndo™ HC spin columns.

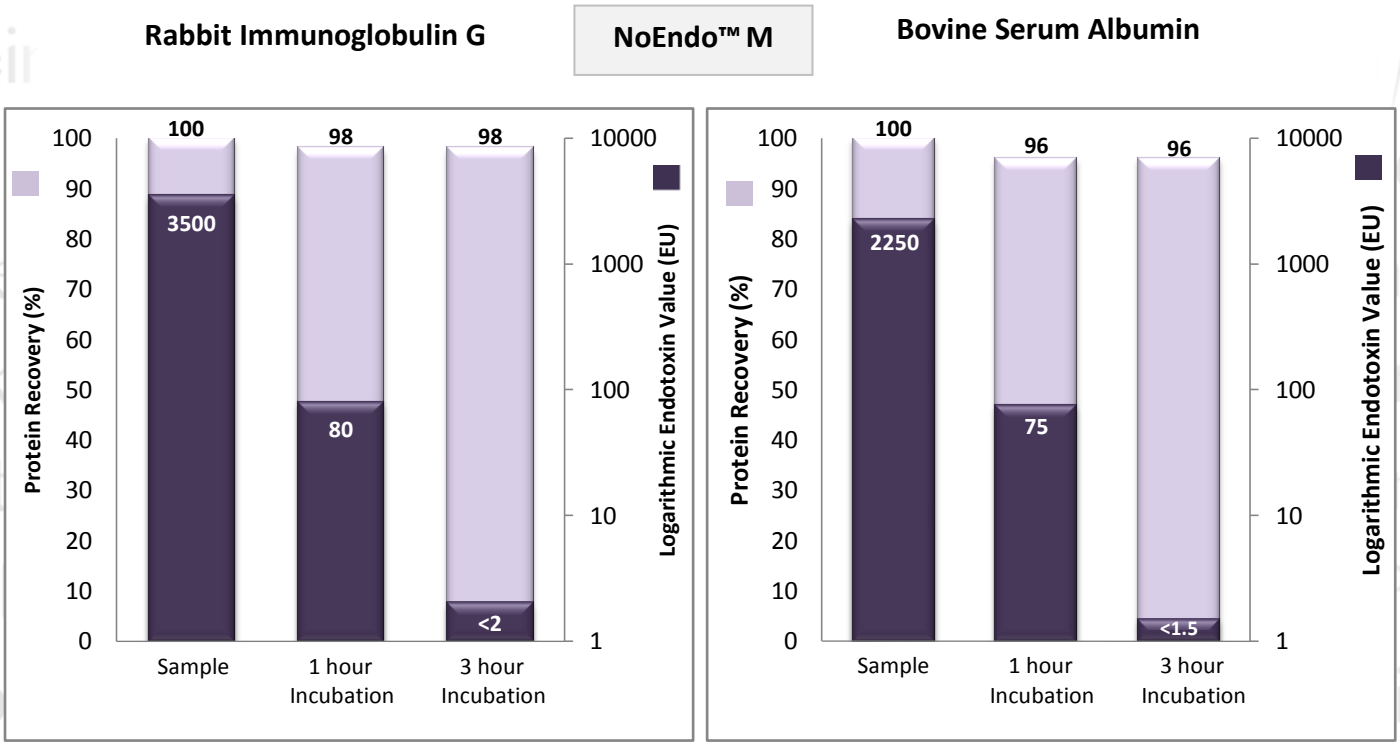


Figure 2: The Proteus NoEndo™ M spin columns effectively removes endotoxin from BSA and rabbit IgG samples (1mg/ml) spiked with *E.coli* lysate. The Proteus NoEndo™ M spin columns were loaded with 0.25 ml NoEndo™ resin and washed at 500 g for 5 min to remove the resin storage buffer. The column resins were then washed with 15 ml equilibration buffer twice. 20 ml protein sample was batch incubated with the washed resin for up to 3 hours on a standard tube roller. The columns were centrifuged at 700 g for 10 min. Endotoxin data was generated using the kinetic chromogenic LAL assay (Charles River Endosafe plate reader). Typically, 3 log reductions in endotoxin were observed.

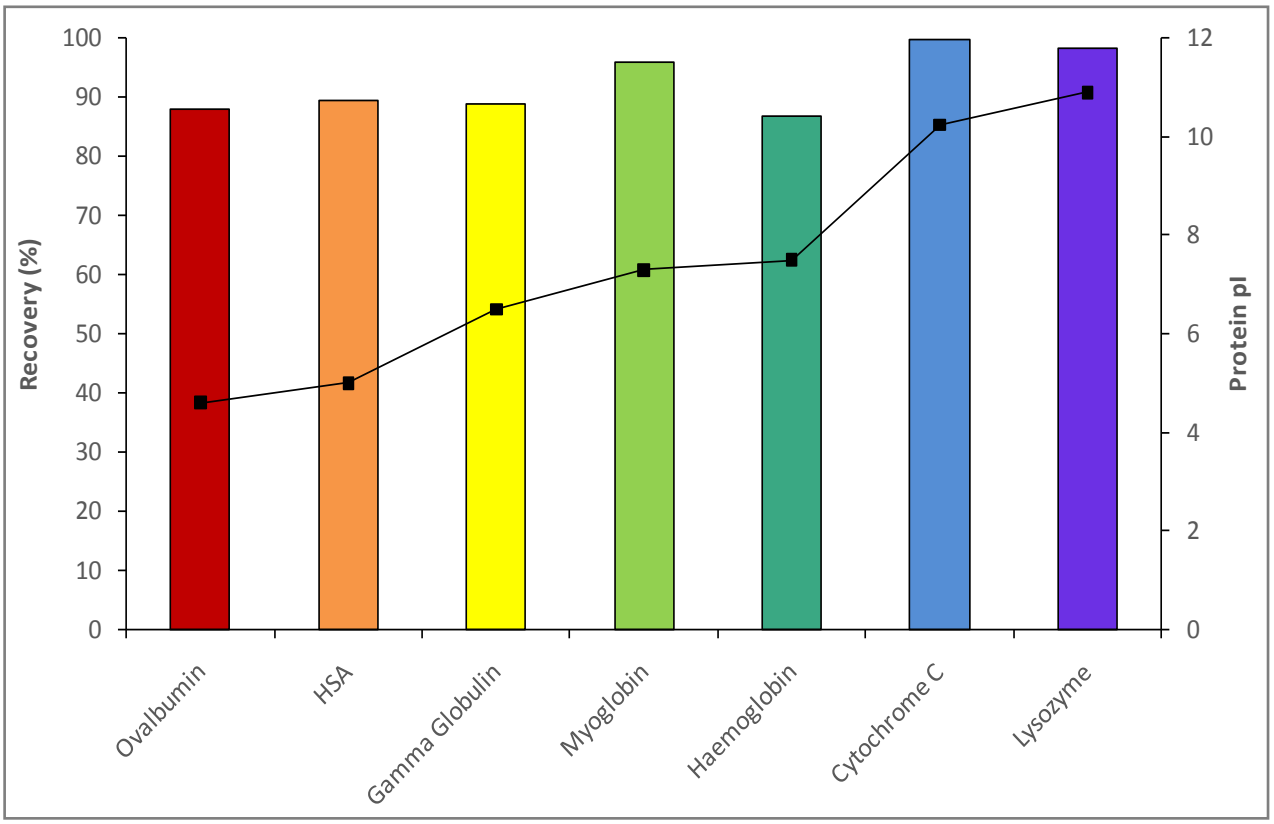


Figure 3: TheProteus NoEndo™ S and Proteus NoEndo™ HC spin columns exhibit low protein binding. The data represents a wide range of proteins that were used through the column regardless of their iso-electric point (pI). Typical protein recoveries close to 90% were obtained.

When to use Proteus NoEndo™ Mini, NoEndo™ Standard or NoEndo™ High Capacity spin columns?

The Proteus NoEndo™ columns have different endotoxin binding capacities. For samples with endotoxin loads less than 3,000 EU, Proteus NoEndo™ M columns are ideal. For samples with endotoxin loads less than 30,000 EU, Proteus NoEndo™ S can be used. For samples with endotoxin loads less than 1,000,000 EU, Proteus NoEndo™ HC columns are required.

Column Specifications

Spin Columns	NoEndo M	NoEndo S	NoEndo HC
Typical *in situ* binding capacity per column	3,000 EU	30,000 EU	500,000-1,000,000 EU
Typical endotoxin binding capacity	300 EU/ml	1,500 EU/ml	30,000 EU/ml
Minimum endotoxin levels tested post-column	<0.03 EU/ml	<0.05 EU/ml	<0.05 EU/ml
Typical endotoxin clearance after 1 pass	-	3 log reduction	3 log reduction
Typical endotoxin clearance after 2 passes	-	4 log reduction	4 log reduction
Typical endotoxin clearance after 1 hour incubation	2 log reduction	-	-
Typical endotoxin clearance after 3 hour incubation	3 log reduction	-	-
Maximum sample load volume	20 ml	20 ml	20 ml
Bed volume	0.25 ml loose	1 ml pre-packed	1.7 ml pre-packed
Resin	NoEndo™ resin	NoEndo™ resin	NoEndo™ resin
Bead size range	100 µm	100 µm	100 µm
Proteus matrix	Cross-linked 6 % agarose	Cross-linked 6 % agarose	Cross-linked 6 % agarose
Recommended working pH	4-8	4-8	4-8
Colour coded end-caps	Supplied in vials	Light green	Dark green

Ordering Information

Kits	Quantity	Product Code
Proteus NoEndo™ M 2 column kit (2 x 0.25 ml resin vials) Proteus NoEndo™ S 2 column kit Proteus NoEndo™ HC 2 column kit Contents – 2 spin columns, 2 centrifuge tubes, 2 resin cartridges/vials	2 M spin columns 2 S spin columns 2 HC spin columns	GEN-NoE2M GEN-NoE2S GEN-NoE2HC
Proteus NoEndo™ M 12 column kit (12 x 0.25 ml resin vials) Proteus NoEndo™ S 12 column kit Proteus NoEndo™ HC 12 column kit (Contents – 12 spin columns, 12 centrifuge tubes, 12 resin cartridges/vials)	12 M spin columns 12 S spin columns 12 HC spin columns	GEN-NoE12M GEN-NoE12S GEN-NoE12HC
Proteus NoEndo™ M 48 column kit (48x 0.25 ml resin vials) Proteus NoEndo™ S 48 column kit Proteus NoEndo™ HC 48 column kit (Contents – 48 Midi spin columns, 48 resin cartridges/vials (excludes 50 ml centrifuge tubes))	48 M Spin Columns 48 S spin columns 48 HC spin columns	GEN-NoE48M GEN-NoE48S GEN-NoE48HC

Loose Resin	Quantity	Product Code
NoEndo™ Resin (25 ml loose resin)	25 ml NoEndo™ resin	GEN-NoE025ML
NoEndo™ Resin (100 ml loose resin)	100 ml NoEndo™ resin	GEN-NoE100ML

Accessories	Quantity	Product Code
<u>Empty spin/batch columns</u> Proteus '1-step batch' Midi spin column pack	 8 pack	 GEN-1SB08
<u>Empty FPLC columns</u> Proteus 1 ml FliQ column Proteus 5 ml FliQ column Proteus 10 ml FliQ column Proteus 20 ml FliQ column	 1 column 1 column 1 column 1 column	 GEN-FliQ1 GEN-FliQ5 GEN-FliQ10 GEN-FliQ20
<u>Empty scalable columns</u> 10 ml Single step column with bottom frit 25 ml Single step column with bottom frit 50 ml Single step column with bottom frit 100 ml Single step column with bottom frit	 10 pack 10 pack 10 pack 10 pack	 9452086-10 9452088-10 9452090-10 9452092-10

Protein Ark Limited

Telephone +44 (0) 33 33 44 20 25

FAX: +44 (0) 33 33 44 20 25

Email: info@proteinark.com