



Proteus NoEndo™ M (Mini) Spin Column Kits

User Guide

Introduction

The Proteus kits are designed for simple, rapid removal of endotoxin from a wide range of protein solutions. Proteus spin columns replace lengthy and expensive chromatographic methods such as phase separation and FPLC®.

There are three versions of the Proteus kits; the Proteus NoEndo™ M (Mini), the Proteus NoEndo™ S (Standard) and NoEndo™ HC (High Capacity) spin column kits which are suitable for different endotoxin loads. For low endotoxin samples (less than 3,000 EU), the Proteus NoEndo™ M columns are ideal. For samples with endotoxin loads less than 30,000 EU, Proteus NoEndo™ S and Proteus NoEndo™ HC columns can be used. For samples with endotoxin loads up to 1,000,000 EU, Proteus NoEndo™ HC columns are required. The Proteus NoEndo™ S and NoEndo™ HC kits incorporate pre-packed NoEndo™ resin plugs in ready-to-use spin columns. Endotoxin removal is achieved in a 50 ml centrifuge tube. The Proteus NoEndo™ S and NoEndo™ HC columns contain a proprietary FlowGo™ technology which regulates sample movement through the high-quality affinity resin matrix. The Proteus NoEndo™ M columns incorporate a proprietary SelfSeal™ membrane technology preventing any sample leaking into the collection tube on an orbital mixer. The yields of a gravity flow are provided with the speed of a spin column.

Large numbers of samples can be processed at the same time. There is negligible hold-up volume; ensuring high solute recovery with minimal nonspecific absorptive losses. Protein samples purified using Proteus spin columns may be used for a wide range of laboratory procedures such as biopharmaceutical preparations for proteins, antibodies and vaccines.

Unique Features of the Proteus NoEndo™ Spin Columns:

- Endotoxin levels as low as 0.05 EU/ml with typical protein recoveries > 95%.
- Proteus NoEndo™ kits are designed to eliminate tedious chromatographic steps normally associated with other affinity chromatography methods.
- The unique SelfSeal™ membrane in a column offers high speed, high capture efficiency and high reproducibility.
- These spin columns are rapid endotoxin removal columns which require minimal preparation time. Loose resin is marketed towards the traditional chromatography market.

- All pre-packed columns have clear and robust specifications generated in situ. No unrealistic static binding capacities are quoted!
- The spin columns allow multiple and parallel rapid purifications. This is ideal for rapid screening and method development.
- The kits offer a standardised method for high grade removal of endotoxin from a broad spectrum of monoclonal antibodies and proteins. All components of the kits will be fully traceable.
- The provision of a disposable spin column is ideally suited to GMP production where current user requirements and price sensitivities demand regeneration of the affinity matrix using harsh treatments.

Contents of Proteus NoEndo™ Kit with NoEndo™ Resin:

Proteus Kit code:	NoE2	NoE12	NoE48
• ProteusNoEndo™ vials containing 0.25 ml NoEndo™ resin	2	12	48
Proteus spin column barrels with clear spin push cap (20ml capacity in a swing bucket rotor)	2	12	48
• Yellow batch incubation screw caps	2	12	48
• 50 ml centrifuge tubes.	2	12	
• Protocol Card	1	1	1

Additional Materials Required:

- Filter units: 0.2 µm syringe filters for clarification.
- Low endotoxin pre-equilibration buffer (PBS recommended).
- 50 ml centrifuge tubes.
- A bench-top centrifuge with swing bucket rotor capable of handling 50ml centrifuge tubes.
- Quartz cuvettes for UV absorbance measurements.
- UV/VIS spectrophotometer.
- Pyrogen-free test tubes, pipettes and buffer for Endotoxin Assay.

Note: The preferred rotor is a swinging bucket rotor. For optimal performance with a fixed angle rotor, ensure that the orientation of the spin column in the rotor is the same for sample binding and washing.

Chemical Compatibility:

Compatible with:	0.5M NaOH PBS buffers Detergents, Urea, guanidinium chloride Water miscible solvents (e.g. 20% ethanol)* Stable between pH 3-13 10 mM ETDA Stable to 2-40 °C, recommended storage temperature 2-8 °C Operational Flow Rates : 200 cm/hr
Incompatible with:	Oxidising agents Strong acids All Tris buffers All Glycine buffers Most buffers with amine groups

* NOTE: Water miscible organic solvents such as ethanol should be introduced at incremental concentrations up to the desired concentration otherwise bed movement in packed columns may occur. The same is true when exchanging from an aqueous organic solvent to an aqueous solution.

Storage Conditions:

- Remove the Proteus NoEndo™ resin vials from the kit and store it at 2-8°C. There is no need to place the rest of the kit in a refrigerator or cold room. Do not freeze the resin vials or store them at room temperature. Freezing the suspension will damage the agarose beads. The resin is pre-swollen and defined. Proteus spin columns are stable for up to 2 years at 2-8 °C from the date of manufacture. The expiration date is recorded clearly on the outside of the pack. All resin is stored in 20% v/v ethanol containing 0.1M NaCl.

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Technical support:

Contact the Protein Ark technical support and sales centre for assistance:

Telephone +44 (0) 33 33 44 20 25

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

Email: info@proteinark.com

Web: www.proteinark.com

Disclaimer:

- This product is for research use only and is not intended for use in clinical diagnosis. No claims beyond replacement of unacceptable material or refund of purchase price shall be allowed.

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

Affinity chromatography:

The essence of affinity chromatography utilizes the concept of bio-specificity, implying an interaction between a natural binding site and the natural ligand, whether it be enzyme substrate, enzyme-inhibitor or resin-endotoxin interactions.

Often a researcher needs to choose the correct base matrix, select the optimal activation chemistry and couple a suitable ligand to develop an affinity column to purify the target protein. Proteus spin columns remove the guesswork associated with optimizing the resin chemistry by offering ready-to-use resin plugs that have satisfied stringent quality control to guarantee reproducible purification process. Many affinity support preparations require less than 5 ml resin so Midi spin columns contain ideal bed volumes.

The columns are normally constructed from polystyrene or polypropylene with a simple barrel and the addition of porous disks to contain the resin. However, the affinity separation often takes several hours to complete; in addition the researcher usually has to pack the column, which can add a minimum 2 further hours to the purification step. The elaborate nature of the packing means that it is usually undertaken by an experienced laboratory worker. We overcome these hurdles with our Proteus NoEndo™ spin columns.

NoEndo™ Resin:

Proteus NoEndo™ M spin columns are designed for the NoEndo™ resin. The NoEndo™ resin is specifically designed with a polyamine chemical synthetic ligand which has high selectivity for endotoxin. This ligand is immobilized on to nearly mono-disperse 6% agarose beads using established chemical methods. It is highly effective in binding and removing endotoxin from a variety of fluids such as water, aqueous solutions and biopharmaceutical preparations such as proteins, vaccines and antibiotics.

The SelfSeal™ Advantage:

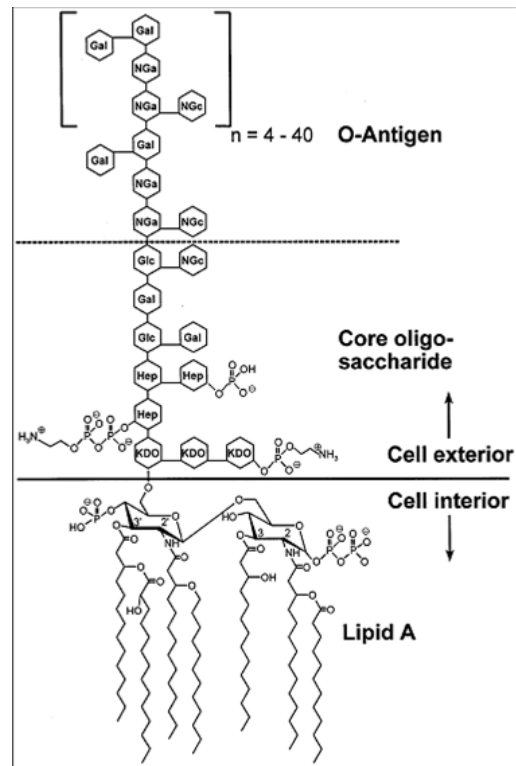
The NoEndo™ M spin columns incorporate our proprietary SelfSeal™ membrane technology. The coated membrane is specially formulated to prevent any sample from leaking into the collection tube on an orbital mixer. Batch incubation can be performed at 4°C and at room temperature. In a centrifuge, the membrane pores dilate and the eluate, free of endotoxin, passes into the collection tube. The contact time is maximized to ensure maximum endotoxin depletion and without losses of protein, antibody or domain antibody.

Background:

Endotoxin are the predominant lipids found in the outer membrane of gram negative bacteria. We know that sub-nanogram levels of endotoxin can trigger immune responses and alter the function of many different cell types and that removal of endotoxin is one of the most difficult downstream processes during protein or antibody purification. Gram negative bacteria are widely used as vectors for the manufacture of recombinant peptides

and proteins. Much work has been spent over the years optimizing *E. coli* as an expression host for proteins from higher organisms. As a result, it is generally recognized that the first attempt to express a recombinant protein uses *E. coli* as the expression host. Phage display, which utilizes Gram negative bacteria, is increasingly used for the manufacture of monoclonal antibodies eg Morphosys, Germany. Recombinant viruses and viral vectors are also currently being developed for therapeutic applications including vaccination, gene therapy e.g. to treat conditions such as heart disease, diabetes, muscular dystrophy and cancer 'virotherapy'. These final products are always contaminated with endotoxin.

Contaminating pyrogens such as lipopolysaccharides or endotoxin present in gram negative bacteria need to be removed from protein, antibody and viral vectors. This makes the final product suitable for animal studies, cell cultures and cell based assays.



Endotoxin are of great concern in the medical device and pharmaceutical industries. 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. Endotoxin concentrations as low as 0.3-0.4 EU/ml can induce pyrogenic shock in mammals.

Many commercially available products are unable to remove endotoxin satisfactorily, or require time-consuming incubation steps. It is well documented that there is widespread dissatisfaction with all known commercial methods to remove endotoxin. Typically, the target protein binds as efficiently to the matrix as the endotoxin and cannot be recovered easily, or both endotoxin and protein elute in the flow-through fractions.

Incumbent technologies include:

1. *Affinity-based methods e.g. Polymyxin B-agarose resin.*
Weaknesses: Poor binding capacity. Very expensive resin. Unreliable. Known to bind target proteins non-specifically.
2. *Non-affinity-based methods e.g. Ion exchange chromatography and ultrafiltration.*
Weaknesses: Empirical design of experiments and prior knowledge of key physical properties of target proteins e.g. isoelectric point, molecular weight is required and often difficult to ascertain. Also requires access to both anion and cation exchange technologies.

3. *Detergent-based methods e.g. Phase separation using Triton X-114.*

Weaknesses: Resultant Triton X-114 resides in the sample. It is very difficult to remove TX-114. Also requires instrumentation to heat to 42°C and for subsequent detergent removal (with inevitable protein losses). Methodology is long-winded and unreliable. The detergent is relatively expensive and would add a significant cost to a manufacturing process. It may also affect the activity of the target protein.

The diversity in the number of methods to remove endotoxin indicates a dilemma in endotoxin removal. Often, endotoxin removal requires more than 1 of the above methodologies used in combination. Each of the above procedures address the problems associated with endotoxin removal in completely different ways. However, none of them has broad applicability. Let's take ultrafiltration as an example. For small proteins, such as myoglobin ($M_r \sim 18,000$), ultrafiltration can be useful to remove large endotoxin aggregates, but not monomeric endotoxin which has a molecular weight between 10 and 20 kDa. With large proteins, such as immunoglobulins, ultrafiltration would not be effective for removing endotoxin aggregates. Usually, the procedures employed for endotoxin removal are unsatisfactory when you assess the following criteria: selectivity, adsorption capacity and recovery of the target species.

High cost has also severely dented widespread use of current endotoxin capture methods. In many cases, complete endotoxin removal is only achieved with large losses in protein yields. More importantly, strong selectivity is required as protein samples are often concentrated to between 5-30 mg/ml, and there is significant evidence showing that endotoxin do bind to proteins. Consequently, reduction or removal of endotoxin to less than 0.3 EU/ml sample (1 ng/mg; 10 EU/mg) is a very challenging task. Note that the term EU describes the biological activity of an endotoxin. E.g. 100 pg of the standard endotoxin EC-5 and 120 pg of endotoxin from *E. coli* O111:B4 have activity of 1 EU.

New fields in science increasingly demand that their raw ingredients are certified endotoxin free, as dictated by European Pharmacopoeia and FDA regulations (Gorbet and Sefton, 2005; Petsch et al, 2000). Many laboratories are forced to allocate resources to run parallel production lines that are endotoxin-free. Successful exploitation of an effective endotoxin removal kit will free up vital resources. Anything that reduces laboratory costs and inconvenience will, therefore, attract considerable interest

General Considerations for selecting Optimal Binding Conditions for the Proteus NoEndo™ kits:

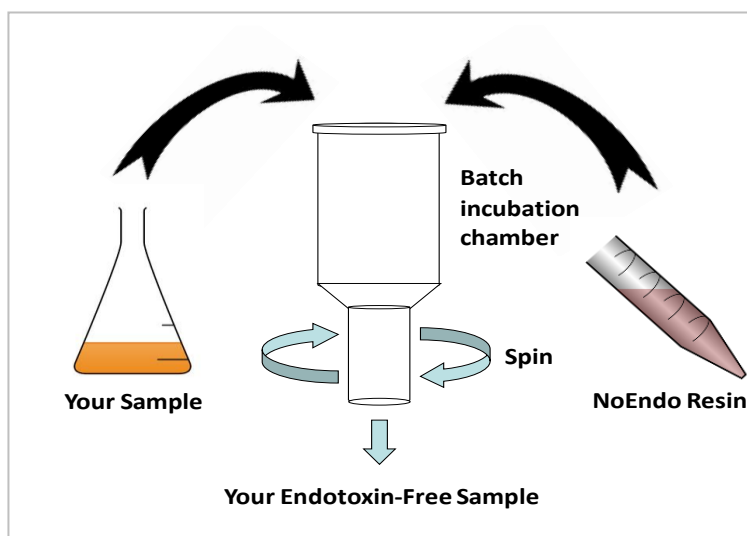
All samples should be filtered just prior to loading even if they have been filtered several days before using the sample. Aggregation/precipitation of proteins is common during storage and repeated freeze-thawing. Protein solution should not be frozen before endotoxin removal. Freeze-thawing increases the formation of endotoxin micelles which can be more difficult to remove.

Binding Kinetics of NoEndo™ Spin Columns:

Controlling the flow rate through an affinity chromatography support is important in achieving binding. Flow rate through the column support is inextricably related to the efficiency of the separation; too fast a flow will cause the mobile phase to move past the beads faster than the diffusion time necessary to reach the internal bead volume. Our studies demonstrate that the large internal surface area of the NoEndo™ resin compensates completely for the velocity of the mobile phase through the column support when the centrifugal speed does not exceed 1,500 x g*. The NoEndo™ resin chemistries used in the Proteus spin column have sufficiently rapid association kinetics between the endotoxin molecule and the immobilised polyamine ligand to allow for optimal diffusional flow through the internal bead structure. Traditionally, gravity flow chromatography is very slow and resolution of the protein separation can be adversely affected by secondary diffusion effects.

* No performance data is available for centrifugal speeds greater than 1,500 x g.

Endotoxin Removal Protocol:



Recommended Protocol:

PRE-EQUILIBRATION

1. Remove the CLEAR spin push cap and pipette 0.5 ml NoEndo™ resin slurry (50% NoEndo slurry: 0.25 ml resin) into the batch incubation chamber of the spin column barrel. Wash the resin at 500 x g for 5 min.
2. Pre-equilibrate the NoEndo™ Mini spin column with 15 ml equilibration buffer by centrifuging the spin column (with the CLEAR SPIN PUSH CAP) at 750 x g for 5 min. It is critical that you repeat this step one more time with a further 15 ml fresh equilibration buffer.

NOTE: If using one spin column, ensure that the spin column is counterbalanced with a unit of equal weight (adjusted with distilled water).

CLARIFICATION OF SAMPLE

3. Pre-filter the sample through a single 0.2 µm (25 mm diameter) syringe filter.

NOTE: As with all forms of chromatography, it is critical that the sample is filtered through a final 0.2 µm syringe filter immediately before loading it on the spin column. Optimal performance of these devices will depend on these instructions being rigorously followed.

SAMPLE LOADING

4. Transfer the spin column barrel to a fresh 50 ml centrifuge tube and load your required volume of filtered sample. The maximum sample volume is 20 ml. Tightly screw the **yellow** batch incubation cap and invert 2-3 times to mix the sample and the NoEndo™ resin. Place the column on a standard tube roller and mix for 1-3 hours. To achieve final endotoxin loads < 0.1 EU/ml from starting loads of 300 EU/ml, we recommend a 2-3 hour batch incubation.

5. After batch incubation, replace the **yellow** cap with the CLEAR spin push cap. Centrifuge the column at 750 x g for up to 10 min and collect the eluate.

NOTE: If using one spin column, ensure that the spin column is counterbalanced with a unit of equal weight (adjusted with distilled water).

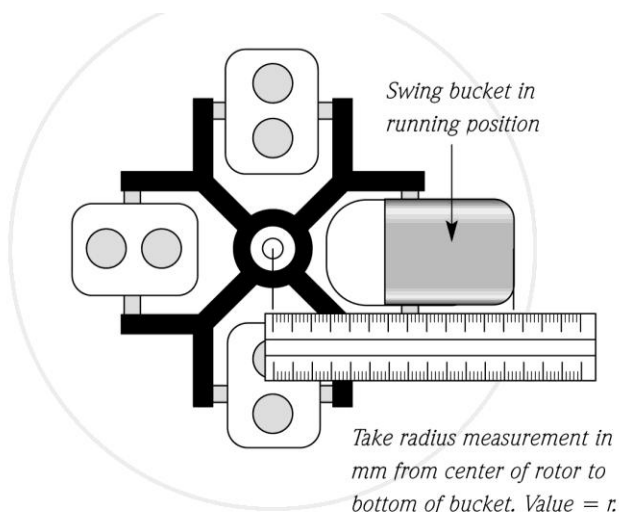
PURIFIED SAMPLE

6. The eluate contains the target analyte largely depleted of endotoxin and is now ready for further downstream analyses.

NoEndo™ and FlowGo™ are trademarks of Protein Ark Limited.

Appendix:

How to convert rpm to g force (RCF) for the MIDI spin column using a swing bucket rotor



It is important that the Proteus spin columns are centrifuged at the correct speeds. Use of higher speeds than those indicated may damage the resin matrix and result in reduced performance. Many centrifuges display only rpm. See the diagram to enable accurate conversion between rpm and RCF (g force). This formula will work on any rotor providing an accurate measurement is taken from the center of the rotor to the bottom of the swing bucket at its open position (when the bucket is rotated through 90° in its running position).

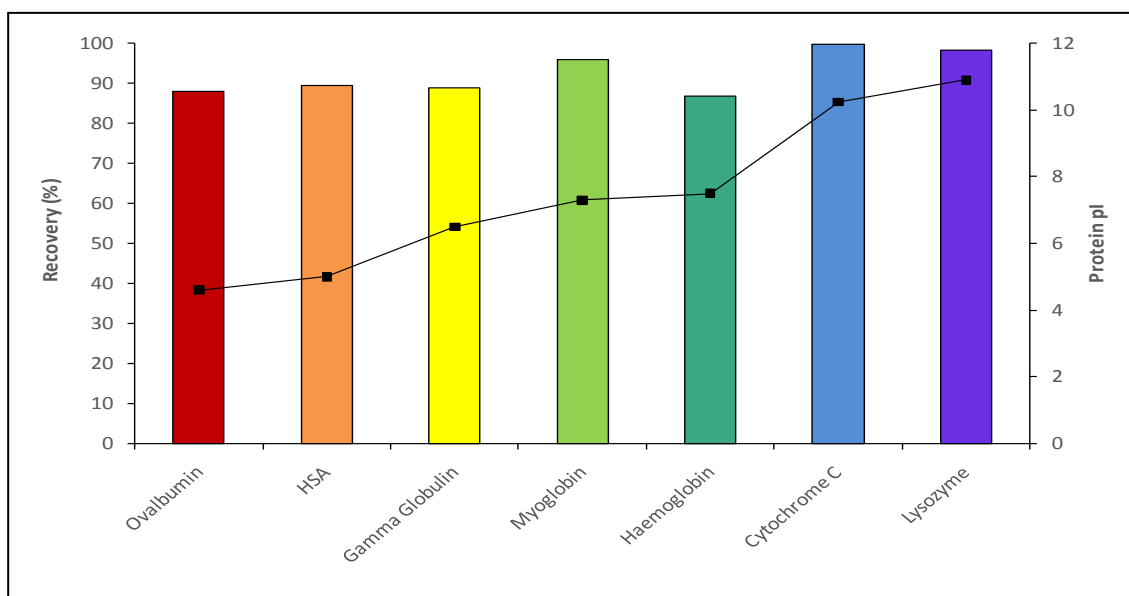
$$RCF = 1.12 \times r \left(\frac{rpm}{1000} \right)^2$$

Eg. 500 g corresponds to 1630 rpm when the radius (r) = 170 mm

Product Performance:

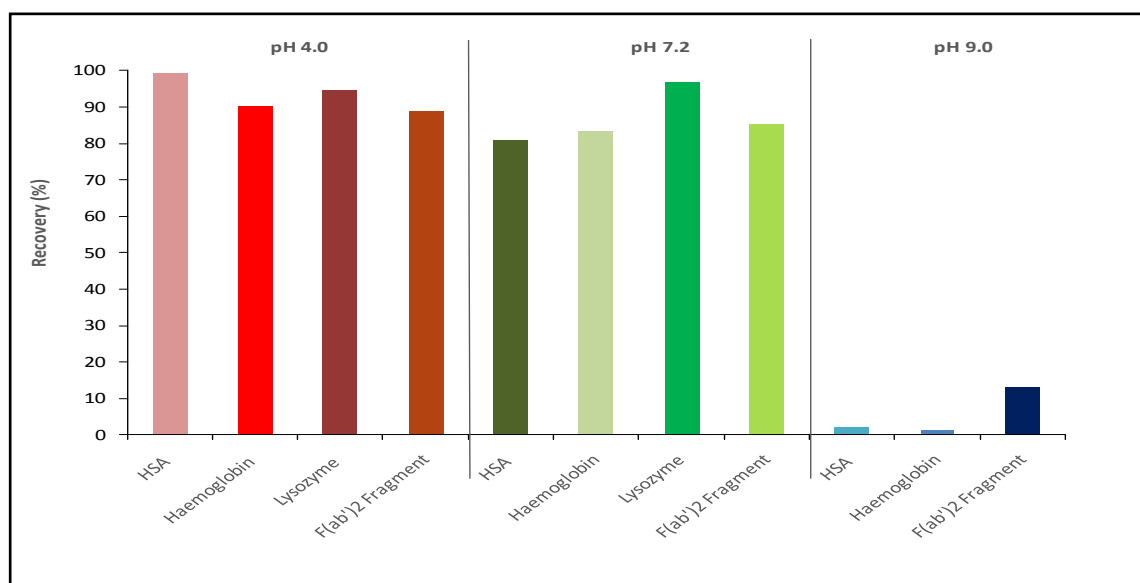
The NoEndo™ resin exhibits low protein binding and a wide range of proteins can be processed regardless of their iso-electric points.

Figure 1: Protein recovery is largely unaffected by NoEndo™ purification.



The Proteus NoEndo™ M, Proteus NoEndo™ S and Proteus NoEndo™ HC spin columns exhibit low protein binding. The data represents a wide range of proteins that were loaded on to the columns regardless of their iso-electric points (pI). Typical protein recoveries close to 90% were obtained.

Figure 2: Protein recovery between pH 4.0 and pH 9.0.



The following proteins were tested in acidic to neutral conditions (pH 4.0 to pH 7.2) using the Proteus NoEndo™ M, Proteus NoEndo™ S and Proteus NoEndo™ HC spin columns. The data here shows that the NoEndo™ resin can operate in these conditions without a

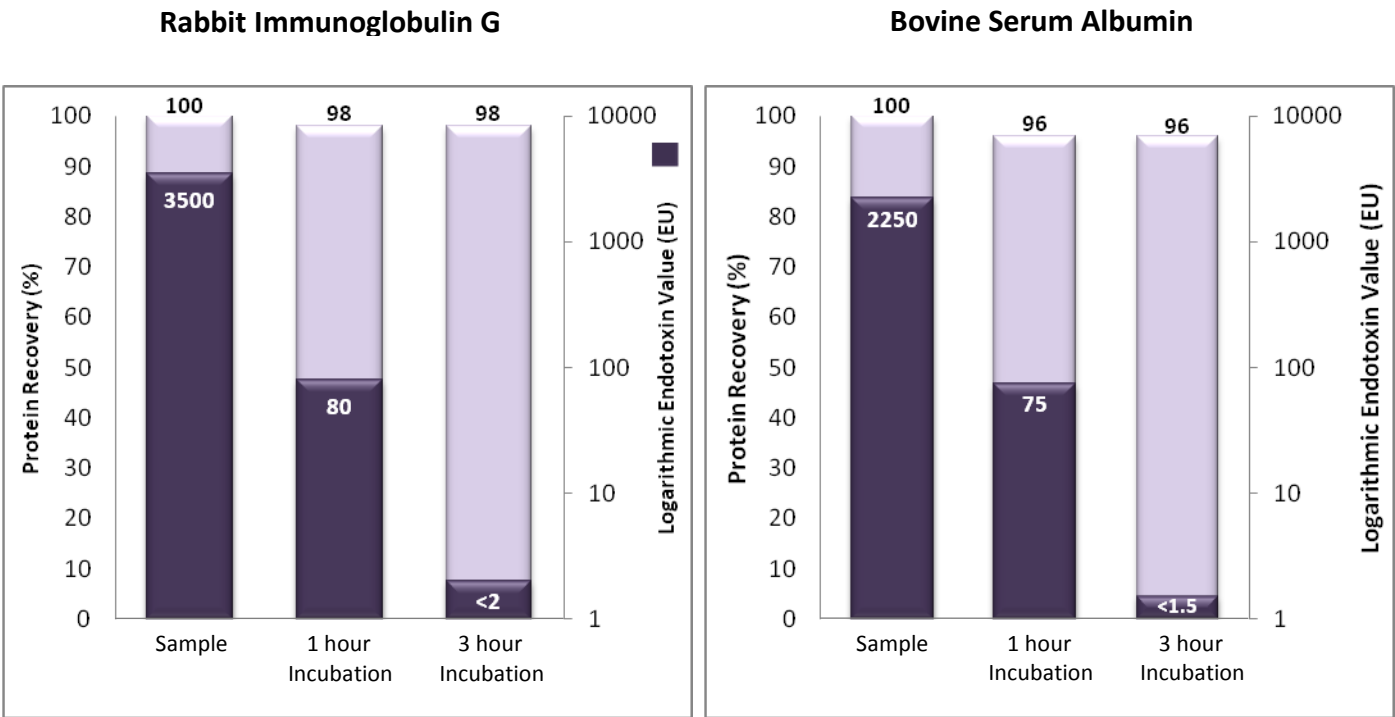
reduction in endotoxin removal and maintaining >85% recovery of various proteins (up to 5 mg/ml). At pH 9.0, both endotoxin and target protein co-bind to the NoEndo™ adsorbent.

Table 1: Endotoxin removal and protein recovery using the Proteus NoEndo™ Midi spin column with BSA spike with *E.coli* lysate.

	Endotoxin removal (EU)	Endotoxin removal efficiency (%)	Protein Recovery (%)
Sample	28,000	-	100
1 st cycle	80	99.7	99
2 nd cycle	0.5	99.9	98.5

The Proteus NoEndo™ S spin column was used to clear endotoxin from a 1 mg/ml BSA sample spiked with *E.coli* lysate. The column was pre-equilibrated with PBS (pH 7.5). After loading 20 ml sample, the column was centrifuged at 100 g for 30 min. The flow through was loaded on to a fresh column and centrifuged using the same conditions. The data represents a typical 4 log reduction in endotoxin load. The protein recoveries were determined separately with the Proteus NoEndo™ spin columns.

Figure 3: Endotoxin removal and protein recovery using Proteus NoEndo™ M spin columns challenged with rabbit IgG or BSA spiked with *E.coli* lysate.



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.

Specifications:

Details	NoEndo™ M Spin Column
Typical <i>in situ</i> binding capacity per column	3,000 EU
Typical endotoxin binding capacity	300 EU/ml
Minimum endotoxin levels tested post-column	<0.03 EU/ml
Typical endotoxin clearance after 1 pass	-
Typical endotoxin clearance after 2 passes	-
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
Bed volume	0.25 ml loose
Resin	NoEndo™ resin
Bead size range	100 µm
Proteus matrix	Cross-linked 6 % agarose

Questions and Answers:

- What is the preferred rotor for the NoEndo™ spin columns?
The preferred rotor is a swing bucket rotor.
- Do I need to filter the buffers prepared in my laboratory?
It is good laboratory practice to filter all buffers.
- Do I need to pre-filter my sample before loading it on to a NoEndo™ spin column?
All samples should be filtered through a final 0.2 µm syringe filter just before the sample loading step.
- What are the typical binding capacities of NoEndo™ Mini spin columns?
The endotoxin binding capacity will critically depend on the resin bed volume. For 0.25 ml resin volume, the typical endotoxin binding capacity is 3,000 EU if the desired final target endotoxin load is <0.05 EU/ml (after 2-3 hour incubation).
- How should I prepare my sample for the NoEndo™ spin column?
Many chromatographic procedures demand that the sample is pre-conditioned prior to loading. It is recommended that all samples are pre-filtered down to a 0.2 µm pore size.

6. What is the maximum volume of solution I can load on to a spin column?
You can load up to 20 ml in a swing bucket rotor and up to 12 ml in a 250 fixed angle rotor.
7. What is the recommended loading buffer?
It is recommended that low-endotoxin PBS buffer be used. Extra care should be taken with buffers containing amines as these interfere with the resin's ability to capture endotoxin.
8. What is the highest speed that I should spin the NoEndo™ M spin column?
There is no need to spin the devices at speeds greater than 1,200 x g. No performance data is available at centrifugal speeds greater than 1,500 x g.
9. Is there a minimum spin speed for the NoEndo™ spin columns?
There are no minimum speeds for NoEndo™ spin columns. The devices can be spun at speeds as low as 50 x g.
10. Why are the sample loading steps for the NoEndo™ M spin columns up to 3 hours?
The NoEndo™ Mini columns incorporate our proprietary SelfSeal™ membrane technology. The coated membrane is specially formulated to prevent any sample from leaking into the collection tube on an orbital mixer. In a centrifuge, the membrane pores dilate and the eluate, free of endotoxin, passes into the collection tube. The contact time is maximised to ensure maximum endotoxin depletion without losses of protein, antibody or domain antibodies.
11. How can I detect endotoxin levels in my sample?
There are many commercially available endotoxin detection instruments available in the market. However, based on test data reliability, use of kinetic chromogenic or turbidometric LAL assay from Charles River Laboratories, Wilmington, MA, US, is recommended.
12. Should I be concerned if the resin dry out during the centrifugal steps?
The NoEndo™ resin is robust and partially dried resin rehydrate rapidly. There are no adverse effects upon the performance of the resin.
13. Can I re-use the NoEndo™ Mini spin columns?
These are disposable columns. Discard the resin after each use!
14. How do I determine the protein recovery?
Protein recovery can be determined by measuring the absorbance of the sample before and after using the Proteus NoEndo™ spin column.
15. My protein sample contains glycerol. Will this affect the performance of the NoEndo™ spin columns?

Glycerol does not impact upon the performance of the NoEndo™ column.

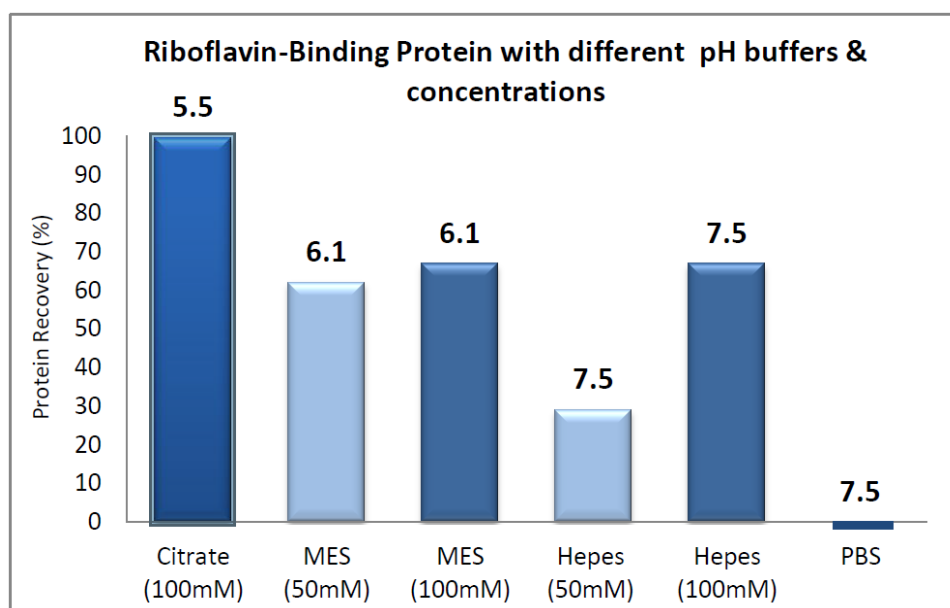
Troubleshooting Assistance

The eluate does not flow easily through the spin column

- Pre-filter the sample just before loading onto the NoEndo™ spin column to prevent the column being clogged with any sample-derived particulates.
- Increase the spin time or spin speed for the elution step.

The recovery of target protein is low

- Some proteins have high affinity for endotoxin.
- There may be interactions between the resin and the protein. If the protein is phosphorylated or acidic, pI of the target protein and the buffer pH needs to be considered. Ideally, the pH of the buffer should be 1 pH unit below the pI of the protein to ensure that the protein has a net positive charge. Data is presented below to show that recovery of an acidic and phosphorylated protein e.g. RBP can be improved by changing the buffer composition.



- The NoEndo™ resin has a cationic functional group which may act as a very weak anionic exchanger at pH values above the pI of the protein. This could lead to slight binding of the target protein which can be minimised by increasing the ionic strength of the buffer up to 0.5 M NaCl.
- Avoid Tris as Tris can cause lower protein recovery.
- The addition of EDTA (up to 20 mM) can enhance protein recovery without affecting endotoxin removal.

Poor resolution of the target protein

- The sample volume or concentration may be too large for the capacity of the resin. In this case, reduce the sample load or sample volume.

- The sample may also need to be filtered carefully.

High levels of endotoxin in my sample

- Avoid freeze-thawing as this increases the formation of endotoxin micelles which can be more difficult to remove.
- 0.1 M glycine can cause minor reductions in endotoxin removal.
- The presence of metal ions (Ca^{2+} , Mg^{2+} and Cu^{2+}) in the sample can hinder the removal of endotoxin.
- Up to 0.3 M NaCl is compatible with endotoxin removal.

Glossary:

Affinity Chromatography – Chromatographic separation based on a specific interaction between an immobilised ligand and a binding site on a macromolecule.

Bed volume – The total volume occupied by the chromatographic packed bed. It is also referred to as the column volume or CV.

Endotoxin – A heat-stable pyrogenic toxin present in the intact bacterial cell. Endotoxins are lipopolysaccharide complexes that occur in the cell wall.

EU/ml – A quantification of endotoxin levels relative to a specific quantity of reference endotoxin. 1 EU/ml is approximately equal to 0.1 ng/ml.

EU - The unit EU (endotoxin unit) describes the biological activity of LPS.

Freeze-thawing – A method that is sometimes used to break open cells by successive periods of slow freezing and thawing. Ice crystals are generated during the freezing stage, which disrupt the cells when they melt during thawing. The method, however, is slow and releases a limited amount of subcellular components.

Isoelectric point – The pH at which the protein has no net charge.

LAL - Limulus amoebocyte lysate (LAL) is an aqueous extract of blood cells (amoebocytes) from the horseshoe crab, *Limulus polyphemus*. LAL reacts with bacterial endotoxin.

Micelles – These are lipid molecules that arrange themselves in a spherical form in aqueous solutions.

Monoclonal Antibodies – Monoclonal antibodies are monospecific antibodies that are the same because they are made by identical immune cells that are all clones of a unique parent cell.

Recombinant Protein -Proteins that result from the expression of recombinant DNA within living cells are termed recombinant proteins.

Turbidometric assay -A method for determining the concentration of a substance in a solution by measuring the loss in intensity of a light beam through a solution that contains suspended particulate matter.

Viral Vectors - Viral vectors are agents commonly used by molecular biologists to deliver genetic material into cells.

References

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Gorbet, M.V., Sefton, M.V. (2005) 'Endotoxin: the uninvited guest', Biomaterials, 26 (34):6811-7.

Magalhaes, P. et al. (2007) 'Methods of endotoxin removal from biological preparations', J Pharm Pharm Sci, 10 (3):388-404.