Proteus Endotoxin Removal Kit Handbook

MIDI Standard Kits (PUR028)

MIDI High Capacity Kits (PUR030)

MINI Spin Column Kits (PUR032)

Contents

These kits are for research use only, and should not be used for diagnostic purposes.

Kit Specifications

The Proteus spin columns have different endotoxin binding capacities:

- For samples with endotoxin loads less than 3,000 EU the MINI columns are recommended.
- For samples with endotoxin loads less than 30,000 EU, both the Standard and High Capacity MIDI columns can be used.
- For samples with endotoxin loads greater than 30,000 EU and up to 1,000,000 EU, the High Capacity MIDI columns Proteus are required.

Kit Property	Standard MIDI Spin Column	High Capacity MIDI Spin Column	MINI Spin Column
Typical *in situ* binding capacity per column	≤30,000 EU	30,000-1,000,000 EU	≤3,000 EU
Typical endotoxin binding capacity (operational flow rate 200 cm/hr)	30,000 EU/ml	450,000 EU/ml	300 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 1hr incubation	-	-	2 log reduction
Typical endotoxin clearance after 3hr incubation	-	-	3 log reduction
Maximum sample load volume	20ml	20ml	20ml
Minimum endotoxin levels tested post-column	<0.05 EU/ml	<0.05 EU/ml	<0.03 EU/ml
Bed volume	1ml	1.7ml	0.25ml loose
Resin	Standard resin Prepacked	High Capacity resin Prepacked	Loose/slurry
Bead size range	100µm	100µm	100µm
Proteus matrix	Cross-linked 6 % agarose	Cross-linked 6 % agarose	Cross-linked 6 % agarose
Recommended working pH	4-8	4-8	4-8
Color coded end-caps	Light green	Dark green	Supplied in vials

Advantages of Proteus Endotoxin Removal Kits

Many commercially available Endotoxin Removal kits, which are based upon non-affinity chromatography methods e.g. Ion exchange chromatography, and phase separation using Triton X-114, are inefficient at removing residual endotoxin contamination from advanced biotherapy products, and require time consuming and expensive affinity steps.

Proteus kits are designed to achieve the quality separation you expect from gravity flow column, combined with the speed and ease-of-use of spin columns, and each kit is standardized for the high grade clearance of endotoxins from recombinant proteins, antibodies and viral vectors.

The use of these spin columns enables the rapid processing of a large number of samples, with negligible hold-up volume, high solute recovery, and minimal nonspecific absorptive losses. MIDI kits combine the convenience of pre-packed chromatography resin plugs and proprietary StableFlo™ technology. MINI kits are supplied with loose resin and incorporate SelfSeal™ membrane technology.

Rapid	▪ 30 minutes removal of >95% endotoxin in one step, using the MIDI spin columns. ▪ The spin columns allow multiple and parallel rapid purifications. This is ideal for rapid screening and method development. ▪ No need for time consuming chromatographic steps normally associated with other affinity chromatography methods.
Effective	▪ Endotoxin levels as low as 0.05 EU/ml with typical protein recoveries > 90-95%. ▪ The unique StableFlo™ and SelfSeal™ membrane technology allows for high speed, high capture efficiency and high reproducibility. ▪ The kits offer a standardized 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.
Convenient	▪ The MIDI pre-packed chromatography resin plugs offer no mess, no filling columns, no pumps, no lengthy steps and minimal optimization required. ▪ The MINI SelfSeal™ Technology allows for minimal manual intervention, high capture efficiency, no dilution of sample, perfect for final polishing steps. ▪ The provision of a disposable spin column is ideally suited where current user requirements and price sensitivities demand regeneration of the affinity matrix using harsh treatments.
Flexible	▪ MINI and MIDI formats available in pack sizes of 12 units

Endotoxin Removal Resin

The proprietary Proteus resin is specifically designed with a polyamine chemical synthetic ligand which has high selectivity for endotoxins. This ligand is immobilized on to a near mono-disperse 6% beads agars using established chemical methods. It is highly effective in binding and removing endotoxins from a variety of fluids such as water, aqueous solutions and biopharmaceutical preparations such as proteins, vaccines and antibiotics.

Endotoxin Removal Protocols

N.B. Protocol cards are supplied with each kit

(i) Proteus MINI Endotoxin Removal Kit

Pre-equilibrium

1. Remove the CLEAR spin push cap and pipette 0.5ml of resin slurry (50% slurry: 0.25ml resin) into the batch incubation chamber of the spin column barrel. Wash the resin at 500xg for 5 min.

2. Replace the CLEAR spin push cap and pre-equilibrate the Mini spin column with 15ml equilibration buffer by centrifuging the spin column at 750 x g for 5 min. It is **critical** that you repeat this step once more with a further 15ml 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

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

   NOTE: As with all forms of chromatography, it is critical that the sample is filtered through a final 0.2µm 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

1. Transfer the spin column barrel to a fresh 50ml centrifuge tube and load your required volume of filtered sample. The maximum sample volume is 20ml. Tightly screw on the yellow batch incubation cap and invert 2-3 times to mix the sample and the 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 2-3 hour batch incubation.

2. After batch incubation, substitute 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

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

(ii) Proteus Endotoxin Removal MIDI kit

After loading the plug into the spin column and placing the spin column into a centrifuge tube, follow the procedure below.

Pre-equilibrium

1. Pre-equilibrate the spin columns with 10ml equilibration buffer by centrifuging the spin column at 500 x g for 3 min.

   NOTE: If using one spin column, ensure that the spin column is counterbalanced with a unit of equal weight (adjusted with distilled water; without a plug).

Clarification of sample

1. Pre-filter 20-25ml sample through a single 1.2µm (25mm diameter) syringe filter to remove any cellular debris. Following this, filter the partially clarified sample through a single 0.2µm (25mm diameter) syringe filter.

   NOTE: As with all forms of chromatography, it is critical that the sample is filtered through a final 0.2µm 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

1. Pipette 20ml sample into the spin column.
   - Centrifuge the spin column at 100 x g for 30 min.
   - Ideal sample loading conditions are obtained using a flow rate of less than 1ml/min. It may be necessary to increase the spin time or spin speed if any sample remains on the top of the plug. Spin speeds as high as 1,500 x g have no damaging effect upon the resin.
   - If the flow rate is slower than expected this may be indicative of a partially clogged plug resulting in incomplete filtration of the sample.

   NOTE: When necessary, perform a further wash step by reloading 1-2ml (Standard column) or 2-4ml (High Capacity column**)** equilibration buffer for maximum protein recovery.

2. For maximal removal of endotoxin, reload the sample into a new spin column (pre-equilibrated as per step 1) and repeat the centrifugation step (100 x g for 30 min). Increase the spin time or speed if any sample remains above the plug.

   NOTE: If using one spin column, ensure that the spin column is counterbalanced with a unit of equal weight (adjusted with distilled water; without a plug).

Purified sample

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

Chemical Compatibility

Compatible with	Incompatible with
0.5M NaOH	Oxidizing agents
All commonly used buffers except for Tris and Glycine	Strong acids
Detergents, Urea, guanidinium chloride	All Tris buffers
Water miscible solvents (e.g. 20% ethanol) *	All Glycine buffers
Stable between pH 3-13	Most buffers with amine groups**
10mM ETDA	

** 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. ** Urea and guanidinium chloride are the exception to this.*

Low protein recovery may indicate interaction between the resin and the protein. If the protein is phosphorylated or acidic, iso-electric points (pI) of the target protein and the buffer pH needs to be considered. Ideally, the pH of the buffer should ideally 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 (see figure 1).

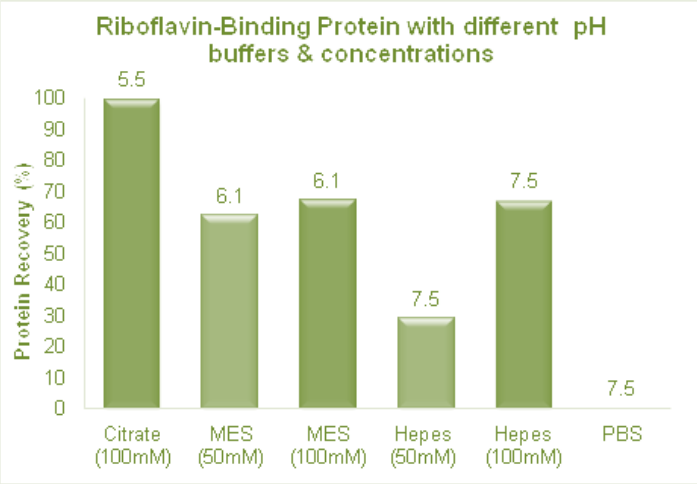


Figure 1 Data showing recovery of an acidic and phosphorylated protein using different buffer compositions

The 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 minimized by increasing the ionic strength of the buffer up to 0.3M NaCl.

The addition of EDTA (up to 20mM) and arginine (0.5M) can enhance protein recovery without affecting endotoxin removal.

Binding Kinetics of Low Endotoxin Spin Columns

The resin chemistries used in these spin column kits have sufficiently rapid association kinetics between the endotoxin molecule and the immobilized 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.

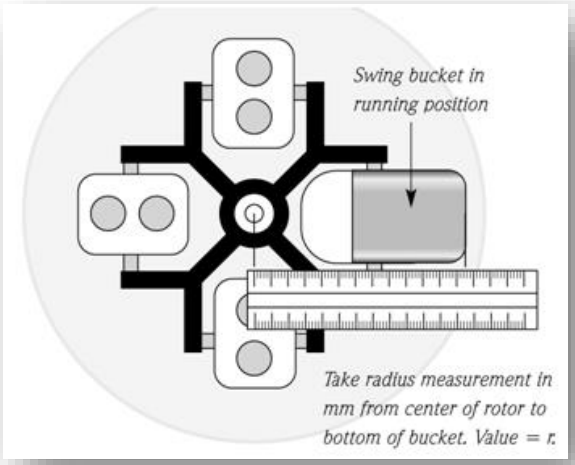
Controlling the flow rate through an affinity chromatography support is important in achieving binding. Flow rate through the column support is inextricably related to the efficiency of the separation; too fast a flow will cause the mobile phase to move past the beads faster than the diffusion time necessary to reach the internal bead volume. Our studies demonstrate that the large internal surface area of the Proteus 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*.

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

Conversion of rpm to g force (RCF) for the Spin Columns 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 x r (rpm/1000)^2



The StableFlo™ Advantage

StableFlo™ is a unique technology which uses back pressure, to enable a steady and controlled flow of sample and buffer through the affinity MIDI 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 SelfSeal™ Advantage

The MINI spin columns incorporate a 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°C and at room temperature. In a centrifuge, the membrane pores dilate and the endotoxin free eluate 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.

Product Performance and Data

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

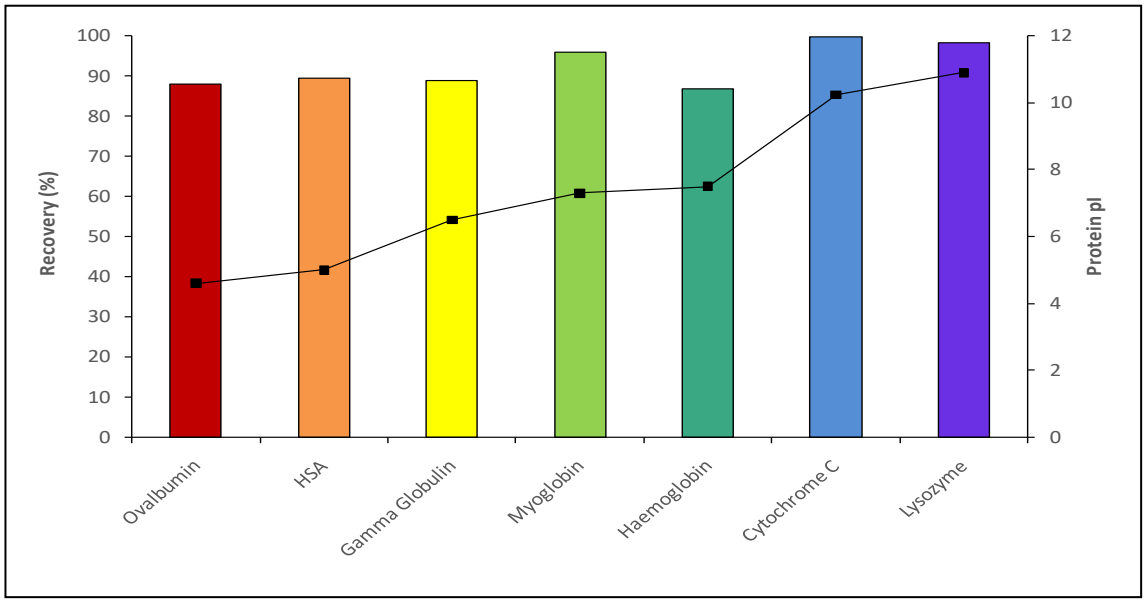


***Figure 2**: Protein recovery is largely unaffected by Low Endotoxin purification.*

The Proteus MIDI and MINI spin columns exhibit low protein binding. The data represents a wide range of proteins that were loaded on to the columns regardless of their pI. Typical protein recovery was 90%

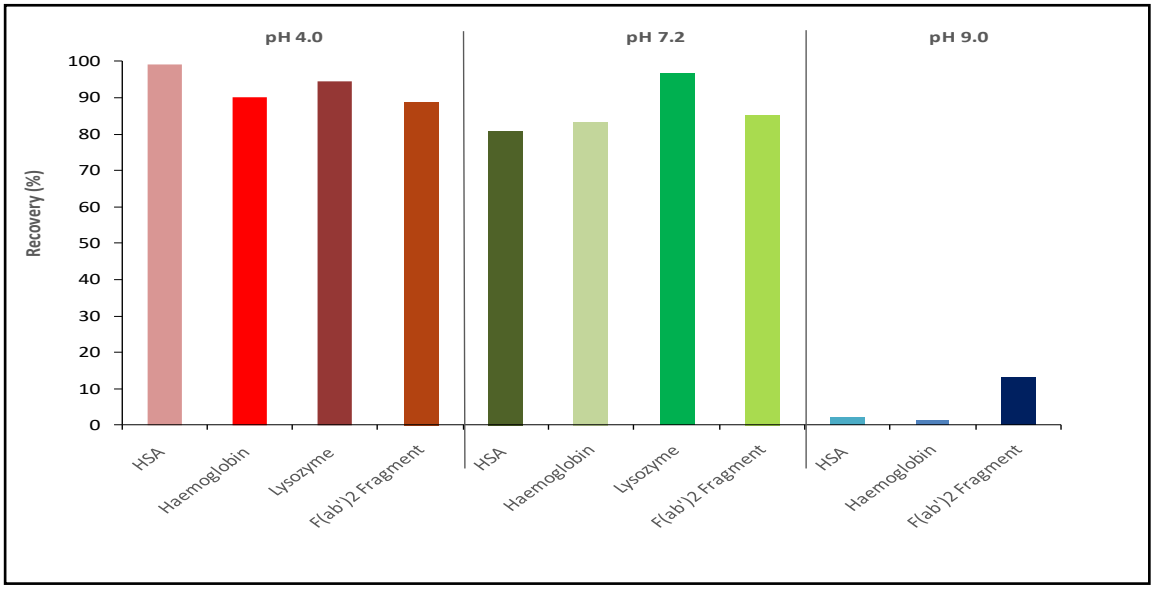


***Figure 3:** Protein recovery between pH 4.0 and pH 9.0.*

The following proteins were tested in acidic to neutral conditions (pH 4.0 to pH 7.2) using the Proteus MIDI and MINI spin columns. The data shows that the resin can operate in these conditions without a reduction in endotoxin clearance and maintaining >80% recovery of various proteins (up to 5mg/mL). At pH 9.0, both endotoxin and target protein co-bind to the adsorbent.

The Standard MIDI spin column was used to clear endotoxin from a 1mg/ml BSA sample spiked with *E.*coli lysate. The column was pre-equilibrated with PBS (pH 7.5). After loading 20ml sample, the column was centrifuged at 100g for 30 minutes. 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 Standard MIDI spin columns (see table 1).

***Table 1**: Endotoxin and protein recovery with BSA spike with E.coli lysate.*

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

The High Capacity MIDI spin columns effectively remove endotoxin from BSA and rabbit IgG samples (1mg/ml) spiked with *E.coli* lysate. The High Capacity spin columns were pre-equilibrated with PBS (pH 7.5) and 20ml protein samples were loaded and centrifuged at 100g for 30 minutes. The flow throughs were loaded on to fresh columns and centrifuged using the same conditions. Typically, a 4 log reduction in endotoxin was observed. The protein recoveries were determined separately with the High Capacity MIDI spin columns (see figures 4a and 4b).

Rabbit Immunoglobulin G

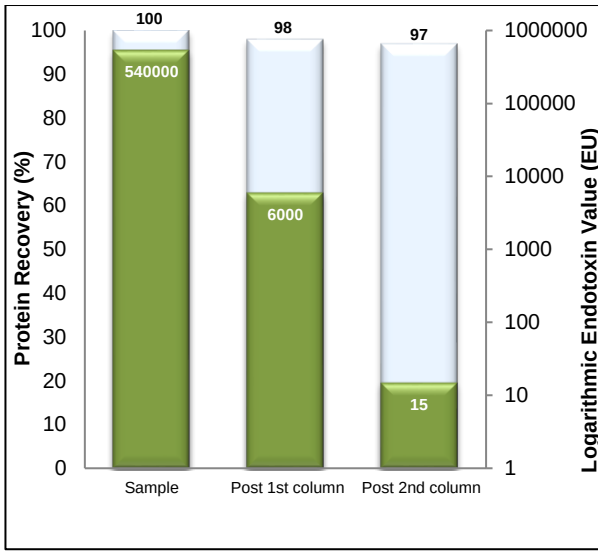


***Figure 4a**: Endotoxin removal and protein recovery using the Proteus High Capacity MIDI spin column sampled with rabbit IgG spiked with E.coli lysate.*

Bovine Serum Albumin

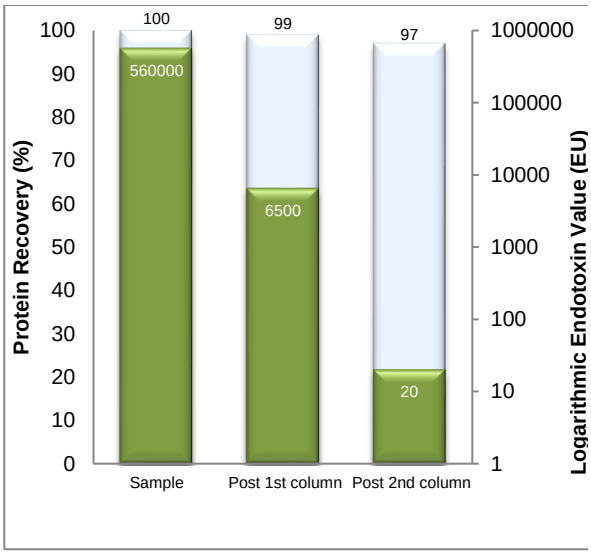


***Figure 4b**: Endotoxin removal and protein recovery using the Proteus High Capacity MIDI spin column sampled with BSA spiked with E.coli lysate.*

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

Rabbit Immunoglobulin G **Bovine Serum Albumin**

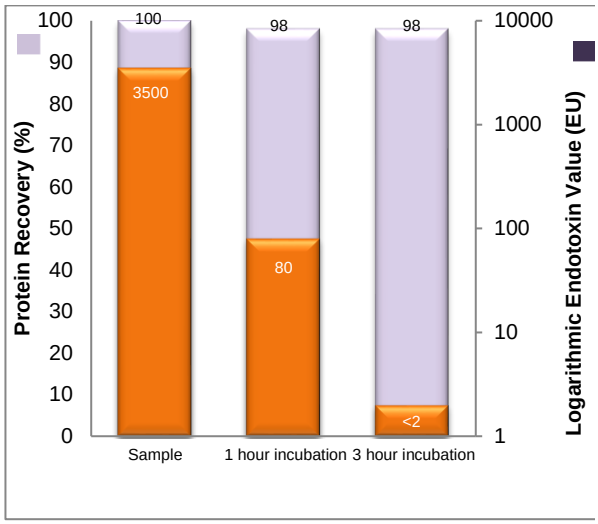


***Figure 5a**: Endotoxin removal and protein recovery using the Proteus MINI spin column sampled with rabbit IgG spiked with E.coli lysate.*

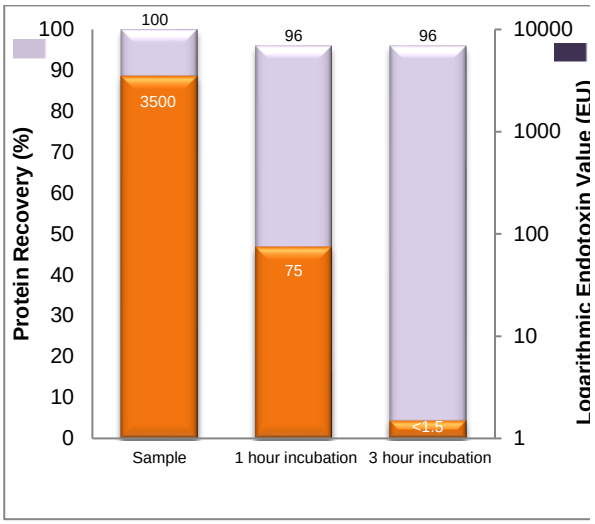


***Figure 5b**: Endotoxin removal and protein recovery using the Proteus MINI spin column sampled with rabbit IgG and BSA spiked with E.coli lysate.*

Frequently Asked Questions

Do samples require filtering before loading?

As a final step all samples should be filtered through a 0.2µm syringe filter immediately prior to loading, even if they have been previously filtered. Aggregation/precipitation of proteins is common during storage. The protein solution should not be frozen before endotoxin removal. Freeze – thawing increases the formation of endotoxin micelles which can be more difficult to remove.

What is the preferred rotor for the spin columns?

The preferred rotor is a swing 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 endotoxin binding, protein elution and washing.

Do I need to filter the buffers prepared in my laboratory?

It is good laboratory practice to filter all buffers.

What are the typical binding capacities of the MIDI and MINI spin columns?

The typical endotoxin binding capacity is 30,000 EU/ml of resin for the Standard MIDI spin column (PUR028), 450,000 EU/ml of resin for the High Capacity MIDI Spin columns (PUR030), and 3,000 EU for 0.25ml resin MINI spin columns (PUR032).

What is the maximum volume of solution I can load on to a spin column?

For both the MIDI and MINI spin columns, you can load up to 20ml in a swing bucket rotor, and for the MIDI spin columns, up to 10ml in a fixed angle rotor. For the MINI spin columns you can load up to 12ml in a 25˚ fixed angle rotor.

What is the recommended loading buffer?

Although all non-Tris and non-glycine buffers can be used, 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.

What is the highest speed that I should spin the columns?

There is no need to spin the devices at speeds greater than 1,000 x g. No performance data is available at centrifugal speeds greater than 1,500 x g.

Is there a minimum spin speed for the MIDI spin columns?

There are no minimum speeds for the spin columns. The devices can be spun at speeds as low as 50 x g.

Why are the samples loading steps for the MIDI spin columns extended to 30 minutes?

The resin plug incorporates a technologically advanced flow regulator which is designed to control the flow rate of the samples through the active column matrix. Observed yields and purities fluctuate as a direct function of the flow rate of the sample loading step. The flow regulator is pre-set to slow down the flow rate to an optimal capture speed. The concomitant increase in the residence time of the endotoxin molecules with the resin in the spin column increases substantially the removal of endotoxin from the target protein. However, unlike many other chromatography systems, there are negligible hold-up volumes and the removal of endotoxin is rapid.

Why are the sample loading steps for the MINI spin columns up to 3 hours?

The 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 maximized to ensure maximum endotoxin depletion without losses of protein, antibody or domain antibodies.

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 we recommend using the kinetic chromogenic or turbidometric LAL assay from Charles River Laboratories, Oregon, US.

Can I autoclave the resin?

No.

Should I be concerned if the resin partially dries out during the centrifugal steps?

The resin is robust, and partially dried resin rehydrates rapidly. There are no adverse effects upon the performance of the resin.

Can I re-use the spin columns?

These are disposable columns where only one sample should be used per spin column.

How do I determine the protein recovery?

Protein recovery can be determined by measuring the absorbance of the sample before and after using the spin column.

My protein sample contains glycerol. Will this affect the performance of the Proteus spin columns?

Glycerol does not impact upon the performance of the spin columns.

Troubleshooting Assistant

The sample does not flow easily through the spin column

- Pre-filter the sample just before loading onto the Proteus spin column to prevent the resin being clogged with the sample.
- Increase the spin time or spin speed for the sample loading step.

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, which increases the formation of endotoxin micelles.
- 0.1M glycine can cause minor reductions in endotoxin clearance.
- The presence of metal ions (Ca^{2+}, Mg^{2+} and Cu^{2+}) in the sample can hinder the clearance of endotoxin. Up to 0.3M NaCl is compatible with endotoxin clearance.

Background Information

Affinity Chromatography and Endotoxins

Endotoxins 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, and the 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. 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.

New fields in science increasingly demand that raw ingredients are certified endotoxin free, as dictated by European Pharmacopoeia and FDA regulations (Gorbet & Sefton, 2005; Petsch *et al,* 2000). Endotoxins 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.

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.

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 (pl), 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 one 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. For small proteins, such as myoglobin

(*M*r ~ 18,000), ultrafiltration can be useful to remove large endotoxin aggregates, but not monomeric endotoxin which has a molecular weight between 10-20kDa.

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.

Many laboratories are forced to allocate resources to run parallel production lines that are endotoxin-free. 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 5-30mg/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 (1ng/mg; 10 EU/mg) is a very challenging task. Note that the term EU describes the biological activity of an endotoxin. e.g. 100pg of the standard endotoxin EC-5 and 120pg of endotoxin from E. coli O111:B4 have activity of 1 EU.

Glossary

Affinity Chromatography
Chromatographic separation based on a specific interaction between an immobilized 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 (pl)
The pH at which the protein has no net charge.

LAL
Limulus amebocyte 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

Persch, D. & Anspach, F.B. (2000) Endotoxin removal from protein solutions. J. Biotechnol. 76 97-119.
Gorbet, M.V. & Sefton, M.V. (2005) Endotoxin: the uninvited guest. Biomaterials. 26: 6811-6817.
Magalhaes, P. *et al.* (2007) Methods of endotoxin removal from biological preparations. J. Pharm. Sci. 10: 388-404.

Ordering Information

Description	Product Code	Quantity
Proteus Low Endotoxin Standard column kit	**PUR028**	**12 spin columns**
Proteus Low Endotoxin High Capacity column kit Contents – 12 MIDI spin columns, 12 resin plugs, 12 centrifuge tubes, 1 insertion tool	**PUR030**	**12 spin columns**
Proteus Low Endotoxin MINI column kit Contents – 12 vials containing 0.25ml resin, 12 MINI spin column barrels with clear spin push cap, 12 centrifuge tubes, 12 Yellow batch incubation screw caps	**PUR032**	**12 spin columns**

Storage Conditions

Upon receipt, remove the Proteus MIDI resin plug box or MINI resin vials from the kit, and store at 2-8˚C. Do not freeze the resin plugs or vials, or store them at room temperature. The remainder of the kit can be stored at room temperature (it does not need to be placed in a fridge or cold room). Freezing the suspension will damage the agarose beads. The resin is pre-swollen and de-fined. Proteus spin columns are stable for up to 2 years at 2-8˚C from the date of dispatch.

Bio-Rad Laboratories
Endeavour House
Langford Lane
Kidlington
Oxfordshire, OX5 1GE

Web: bio-rad-antibodies.com

For Technical support:
Tel: +44 1865 852 733
Fax: +44 1865 852 739
Email: antibody_tech_uk@bio-rad.com