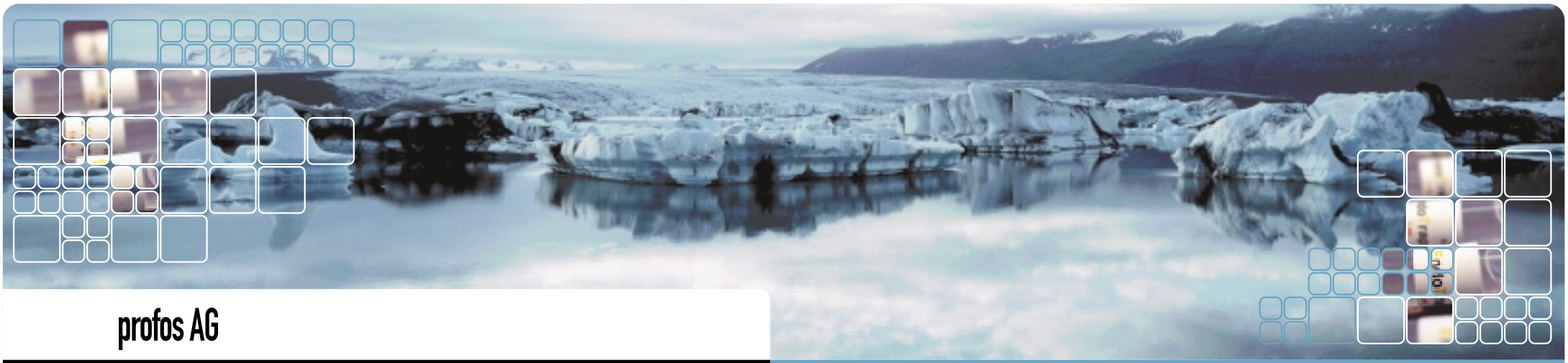
0E.03
product information



profos AG

creative bioscience solutions

profos AG is a reliable and steadily growing biotechnology company. The combination of science, creativity and versatility under the paradigm of efficiency are our philosophy.

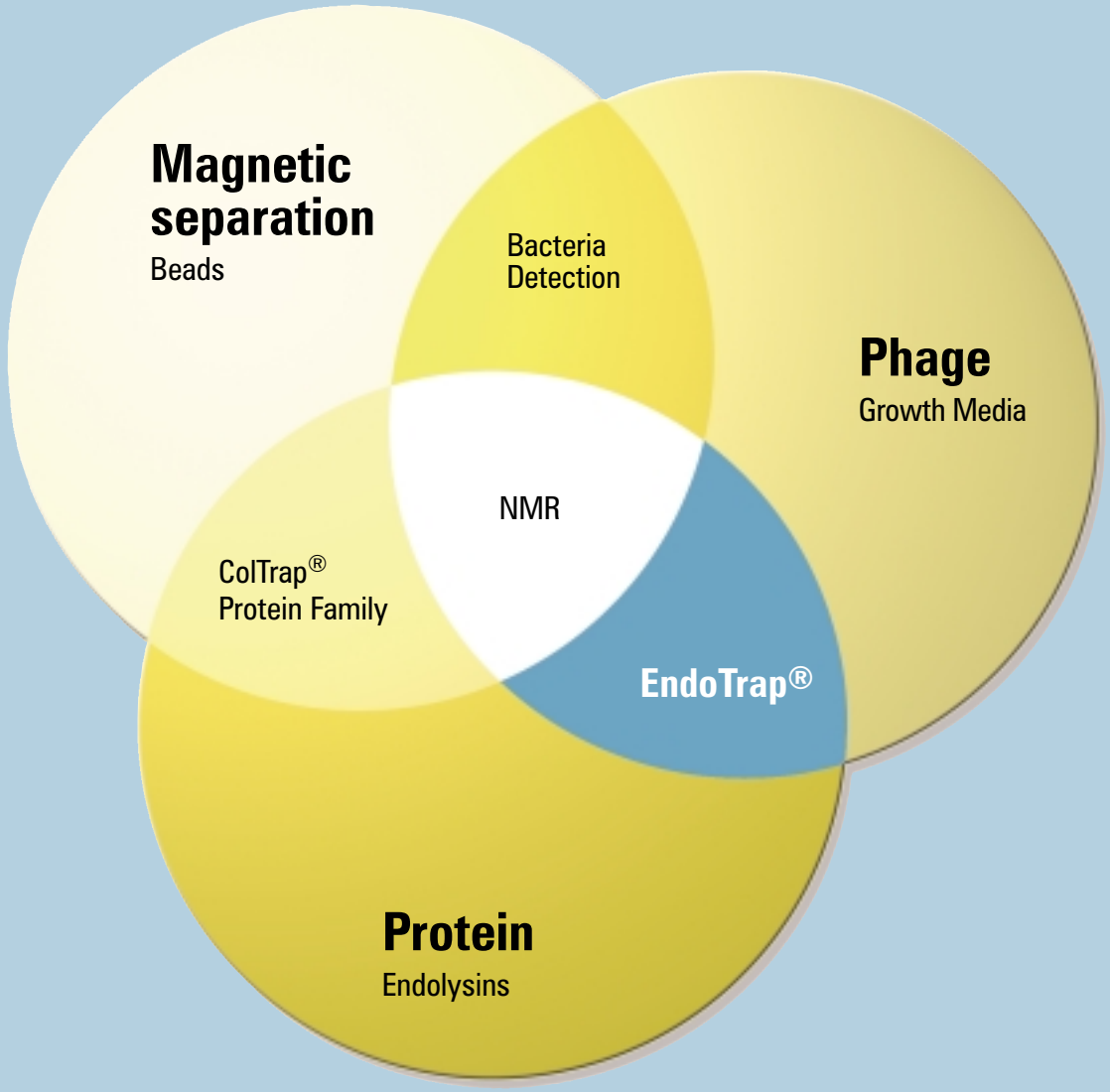
Our products for fast and sensitive detection and isolation of bacteria together with molecular biology products aim at pharmaceutical, food/feed and biotech markets.
Patented technologies enable profos AG to efficiently utilize the interaction of naturally occuring bacteriophages and bacteriophage proteins with their bacterial hosts for development of our products.

Profos' core technologies

A perfect fit for **your** needs

Profos' core competence of combining bacteriophage technology and ligand coupling leads to innovative products for highly selective detection, separation, and harvesting of bacteria ready for further downstream processing.

Additionally, bacteriophages' "natural power" of binding to bacterial surfaces is utilized for highly specific and selective removal of endotoxin in protein purification and downstream processing.



EndoTrap®: highly specific endotoxin removal without incubation

Removal of endotoxin is one of the most difficult downstream processes during protein purification. Many commercially available products are unable to remove endotoxin satisfactory, or require time consuming incubation steps. In many cases, complete endotoxin removal is only achieved with massive substrate loss.

Removal of endotoxin below the detection limit is not only a problem of detection. More importantly, strong selectivity is required. Common late downstream protein solutions are concentrated between 0,1 - 50 mg/ml. Reduction or removal of endotoxin to less than 1 ng/mg (10 EU/mg) is a very difficult task. By using selective sorbents, endotoxin removal from proteins has clear limits. Only methods with highest endotoxin removal capacity combined with excellent recovery rates of the target substance are reasonable and acceptable.

To meet exactly these most challenging requirements, profos AG has developed **EndoTrap®**.
EndoTrap® is based on affinity chromatography by a new, extremely specific ligand for efficient removal of endotoxin without incubation. Extreme ligand stability combined with a sepharose matrix for lowest unspecific substrate binding guarantee highest protein and other compound recovery rates.

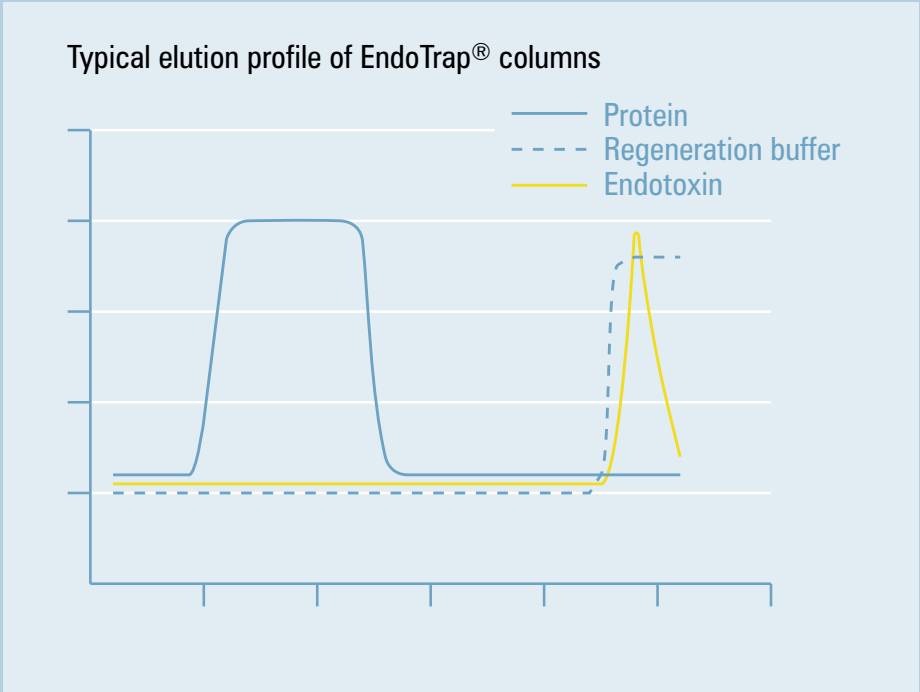


Figure 1: Elution scheme of EndoTrap® columns
Applied protein (blue) elutes immediately after the column void volume. Endotoxin (yellow) stays bound until eluted by a purge with regeneration buffer (dashed blue).

Endotoxin binding

The following pages will give a short introduction in EndoTrap®'s performance and capabilities.

The extremely low binding constant (Kd) of EndoTrap® for endotoxin enables ultrafast and specific endotoxin removal from aqueous solutions such as buffers, DNA, and especially protein samples.

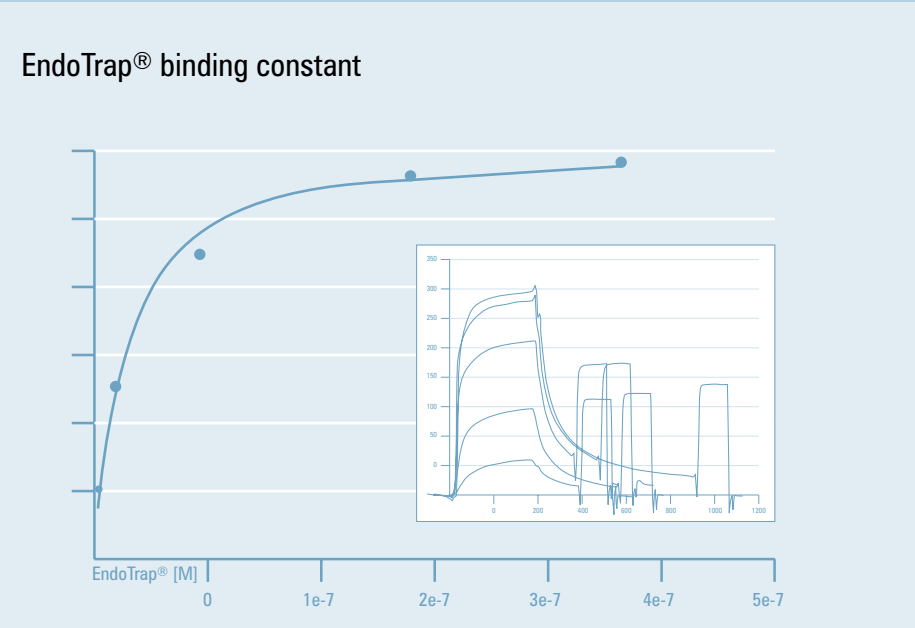


Figure 2: EndoTrap® binds strongly to bacterial LPS
Kd measurements were performed by Surface Plasmon Resonance spectroscopy (SPR). *E. coli* O55:B5 lipopolysaccharide (LPS) was immobilized on a SPR biochip, and increasing concentrations of EndoTrap® ligand were introduced to determine the binding constant of EndoTrap® for LPS. The Kd of EndoTrap® for LPS was determined to be Kd = 5 x 10^{-8} M.

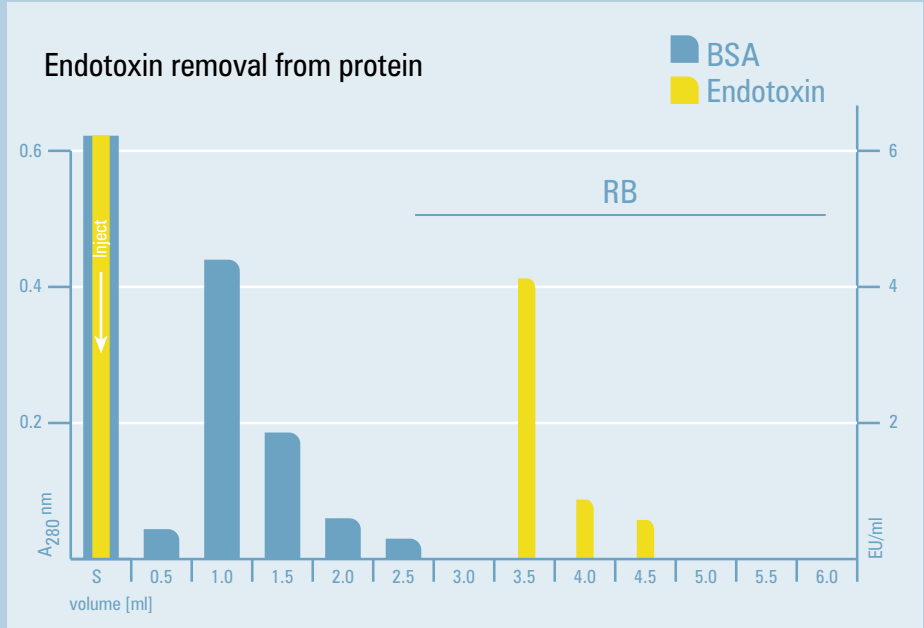


Figure 3: Efficient endotoxin removal from a BSA solution
1 ml Bovine Serum Albumine (BSA) in EndoTrap® equilibration buffer EB (1 mg/ml, blue bars) was spiked with endotoxin (*E. coli* O55:B5, yellow bars) and applied onto a 1 ml EndoTrap® column. Endotoxin free BSA elutes within the first 2.5 ml of flowthrough, endotoxin elutes only after introduction of EndoTrap® regeneration buffer RB.

pH range and buffer compatibility

EndoTrap®'s performance is excellent over a very broad pH spectrum and ensures outstanding removal rates from pH 9 to pH 4, where other systems fail due to their mode of action based on ionic interaction with LPS.

This, combined with EndoTrap®'s usability in most commonly used buffer systems makes EndoTrap® the most versatile endotoxin removal system known.

pH range of endotoxin removal

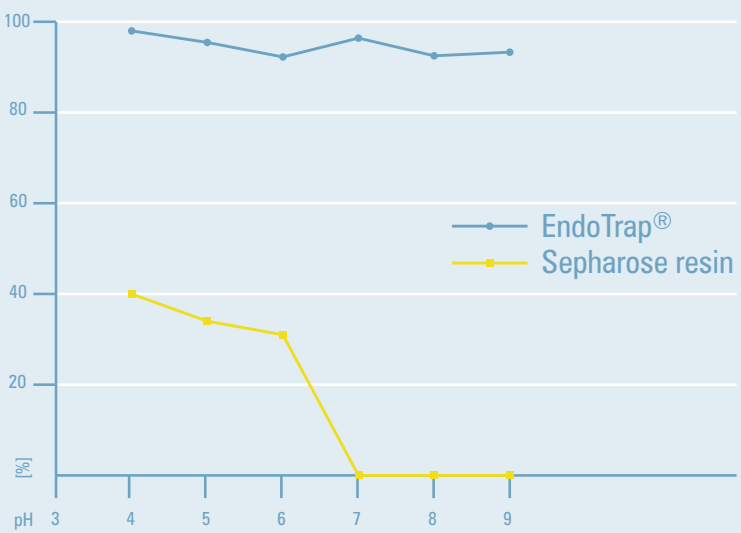


Figure 4: Endotoxin removal is highly efficient over a wide pH range of 4 – 9
AMTC buffer mix (20 mM each of Acetate, MES, Tris, and CAPSO, 150 mM NaCl, 0.1 mM $CaCl_2$) was spiked with endotoxin, and endotoxin removal by EndoTrap® was evaluated. EndoTrap® (blue line) removes endotoxin very efficiently over the pH range of 4-9. The yellow line shows a control of Sepharose resin without ligand.

Buffer spectrum for endotoxin removal

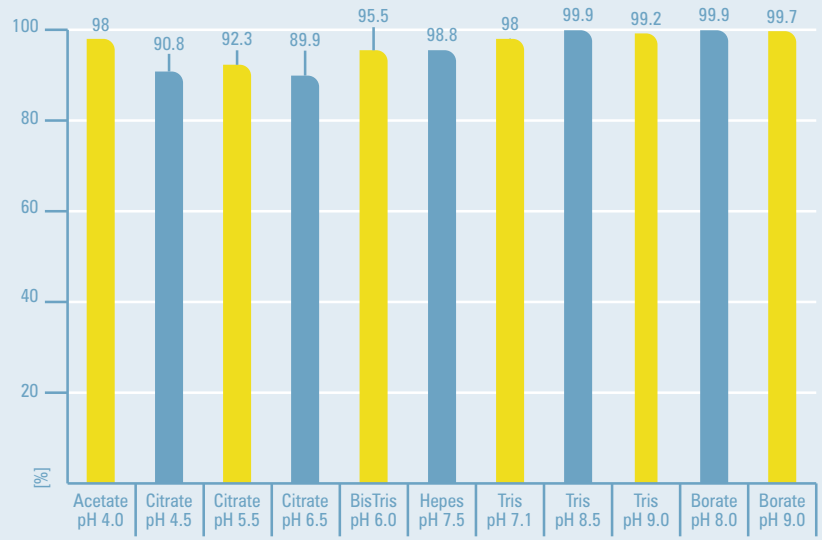


Figure 5: Endotoxin removal from various buffer systems
1 mg/ml solutions of either BSA (Acetate, Citrate, Borate) or Lysozyme (Tris, Bis-Tris) were spiked with endotoxin, and endotoxin removal by EndoTrap® was measured. Endotoxin removal from protein in acidic buffers such as Acetate, pH 4.0, yields similar reduction rates as removal from proteins in strongly basic buffers like Borate, pH 9.0.

Application range and lipopolysaccharide spectrum

A strong binding constant and biochemical inertness make EndoTrap® very widely applicable with proteins of very different biophysical properties. EndoTrap® is highly efficient for LPS from *E. coli*, the most commonly used organism for recombinant protein production. However, LPS from many water contaminating bacteria is also recognized and bound by EndoTrap®.

Certain bacterial species can occur in laboratory water supplies, and are common sources for endotoxin contamination during downstream protein purification and molecular biology processes. LPS from most of these water contaminants is efficiently recognized and removed by EndoTrap®.

Endotoxin removal from various sources

Sample	Isoelectric point pI	Endotoxin removal [%]	Substrate recovery [%]	Endotoxin start contration [EU/ml]	Protein concentration [mg/ml]
Fetuin	4.7	97	100	1,100	2.4
BSA	5.8	99.8	100	1,000	1
Carbonic Anhydrase	7.9	93	99	500	1
Therapeutic protein A	8.5	73	100	4.3	1.4
Therapeutic protein B	9.3	96	98	500	1
Lysozyme	9.4	99.9	100	1,000	1
Polyclonal antiserum		98.8	100	100,000	2
Plasmid (pET21a)		99.9	94.4	1,000	0.05

Table 1: Endotoxin removal from various proteins and DNA
Protein or DNA was spiked with endotoxin in EndoTrap® equilibration buffer EB and subjected to EndoTrap® treatment. In all cases, excellent substrate recovery and endotoxin removal rates could be achieved. Proteins from various sources and of very different biophysical properties were efficiently detoxified by EndoTrap® treatment. EndoTrap® can also be efficiently applied to detoxification of DNA and other nucleid acids.

Wide range of organisms

Lipopolysaccharide from	Endotoxin removal [%]
Escherichia coli O55:B5	99.90
Escherichia coli K12 W3110	99.50
Pseudomonas aeruginosa rough mutant	99.87
Pseudomonas aeruginosa smooth mutant	97.30
Pseudomonas stutzeri	99.50
Citrobacter freundii	99.85
Citrobacter koseri	99.80
Enterobacter aerogenes	99.77
Enterobacter asburiae	99.70
Enterobacter cloacae	98.40
Aeromonas hydrophilia	98.00

Table 2: Binding of LPS from different organisms
LPS was isolated from a variety of gram negative bacteria. Binding of these lipopolysaccharides to EndoTrap® was monitored by application of each LPS sample to a 1 ml EndoTrap® column in EndoTrap® equilibration buffer EB. Endotoxin binding rates averaged 99.3 % for LPS of the strains indicated.

Ionic strength and denaturants

EndoTrap® binds to lipopolysaccharides even under strongly non-physiological conditions. The EndoTrap® ligand's LPS affinity is unaltered by increasing ionic strength, or the presence of high concentrations of chaotropic denaturants such as urea, respectively.

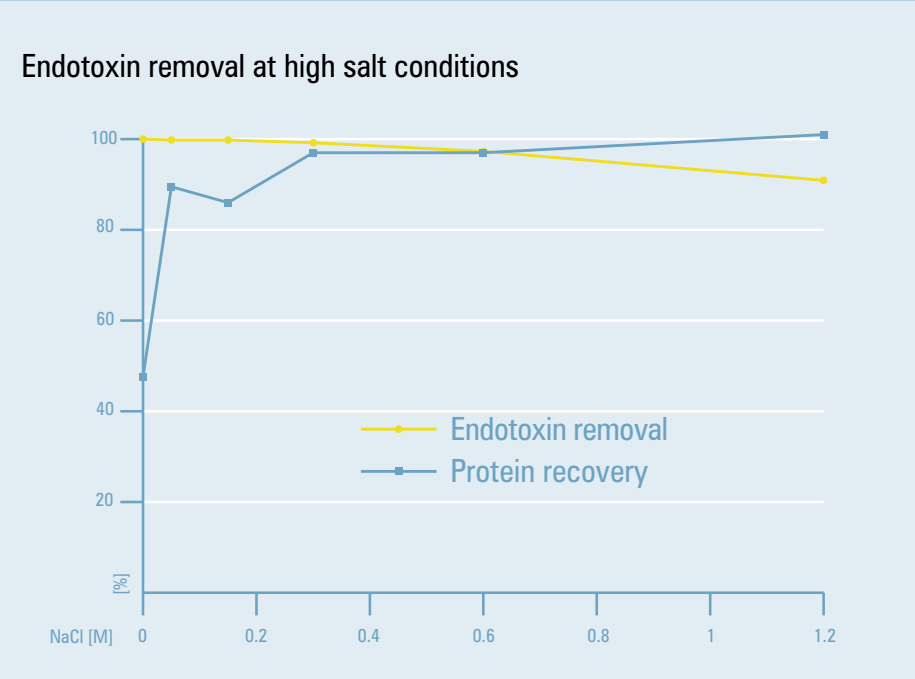


Figure 6: Efficient endotoxin removal at high ionic strength
Endotoxin spiked BSA (1 mg/ml) was incubated in EndoTrap® equilibration buffer EB with increasing concentrations of NaCl and detoxified by EndoTrap®. Protein recovery (blue line) increases with increasing ionic strength. Endotoxin removal (yellow line) is extremely efficient even at 600 mM NaCl, and only slightly decreases at 1.2 M NaCl.

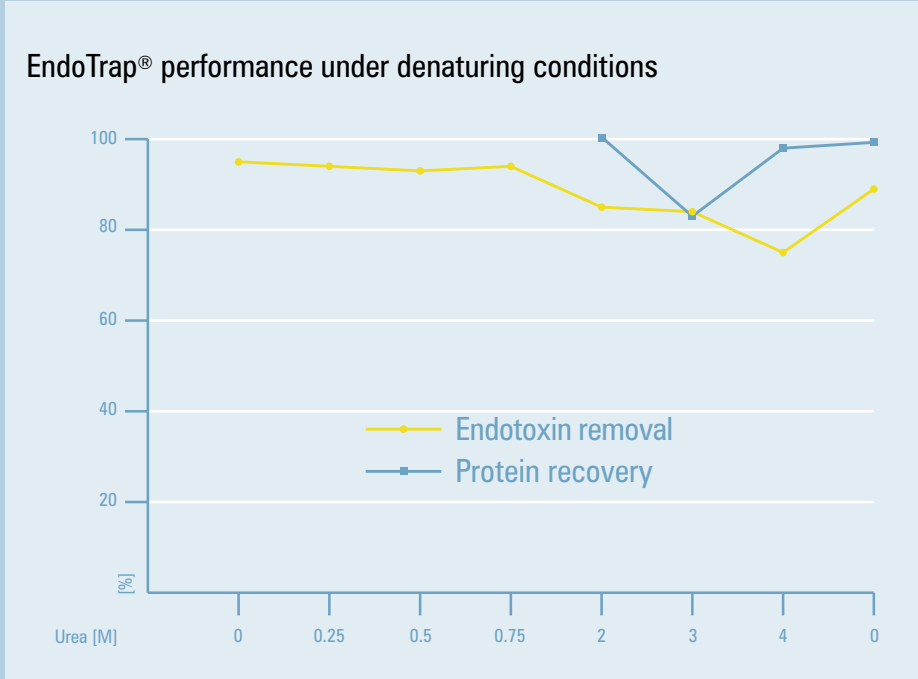


Figure 7: EndoTrap® is still active under chaotropic salt conditons
BSA (1 mg/ml in EndoTrap® equilibration buffer EB plus urea) was spiked with endotoxin, and detoxified by EndoTrap® in the same buffer. Endotoxin removal (yellow) and protein recovery (blue) were monitored. Endotoxin removal and protein recovery only slightly changed with increasing concentrations of urea, and reached near native levels again after re-equilibration in urea-free buffer.

Flow rates and volume loads

For the EndoTrap® affinity based endotoxin removal system, a minimum contact time of one minute must be assured to guarantee optimal performance. Within this minimum contact time, EndoTrap® is independent of flow rate or sample volume. Therefore, EndoTrap® is a universally applicable tool for all systems and sample loads.

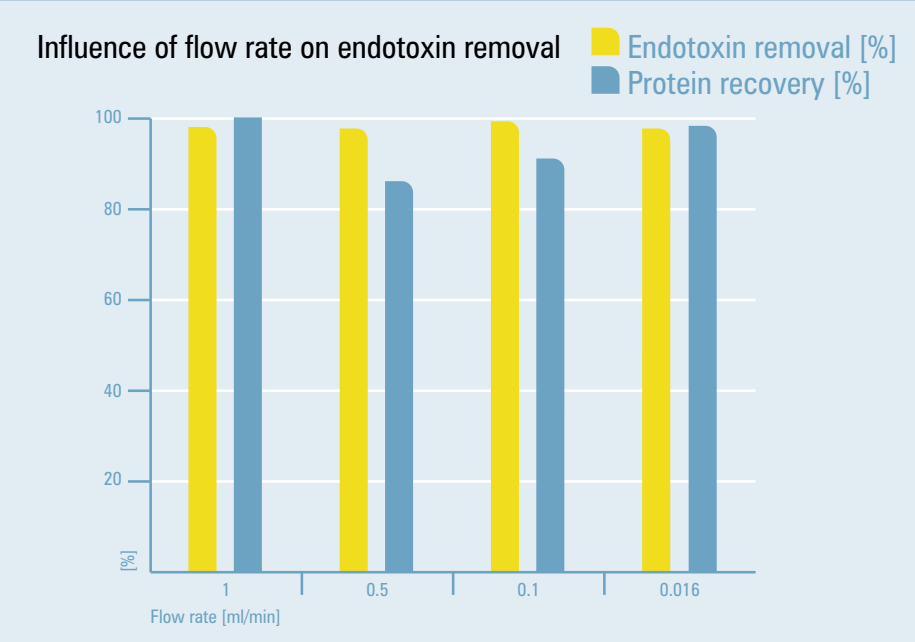


Figure 8: EndoTrap® at various flow rates
Carbonic Anhydrase (0.7 mg/ml) was spiked with endotoxin (1000 EU/ml) and incubated in EndoTrap® equilibration buffer EB. Aliquots were passed over EndoTrap® columns at flowrates of 1 ml/min, 0.5 ml/min, 0.1 ml/min and 0.016 ml/min, respectively. Endotoxin removal and protein recovery were analysed. Both endotoxin removal (yellow bars) and protein recovery (blue bars) were nearly unaffected by the flow rate.

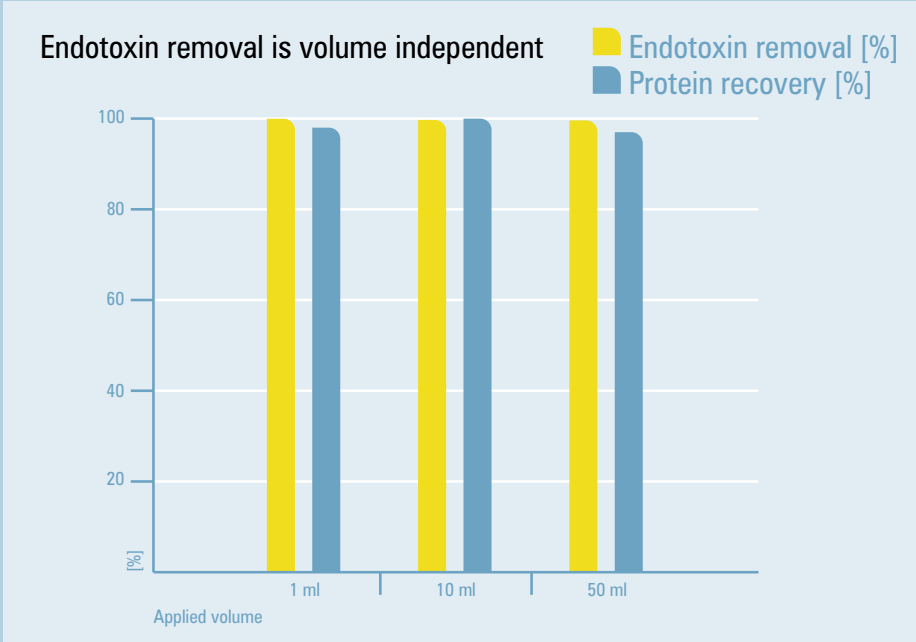


Figure 9: Endotoxin removal efficiency is independent of the loaded volume
BSA (1 mg/ml) was spiked with endotoxin (1000 EU/ml) and incubated in EndoTrap® equilibration buffer EB. Volumes of 1 ml, 10 ml and 50 ml were passed over 1 ml EndoTrap® columns, and endotoxin removal and protein recovery were analysed. Protein recovery (blue bars) and endotoxin removal efficiency (yellow bars) were unaltered even at a volume: resin ratio of 50:1.

Protein concentration and endotoxin contamination range

EndoTrap® efficiently removes endotoxin even from highly concentrated protein solutions. Endotoxin removal efficiency is independent of endotoxin contamination levels, ranging over several orders of magnitude.

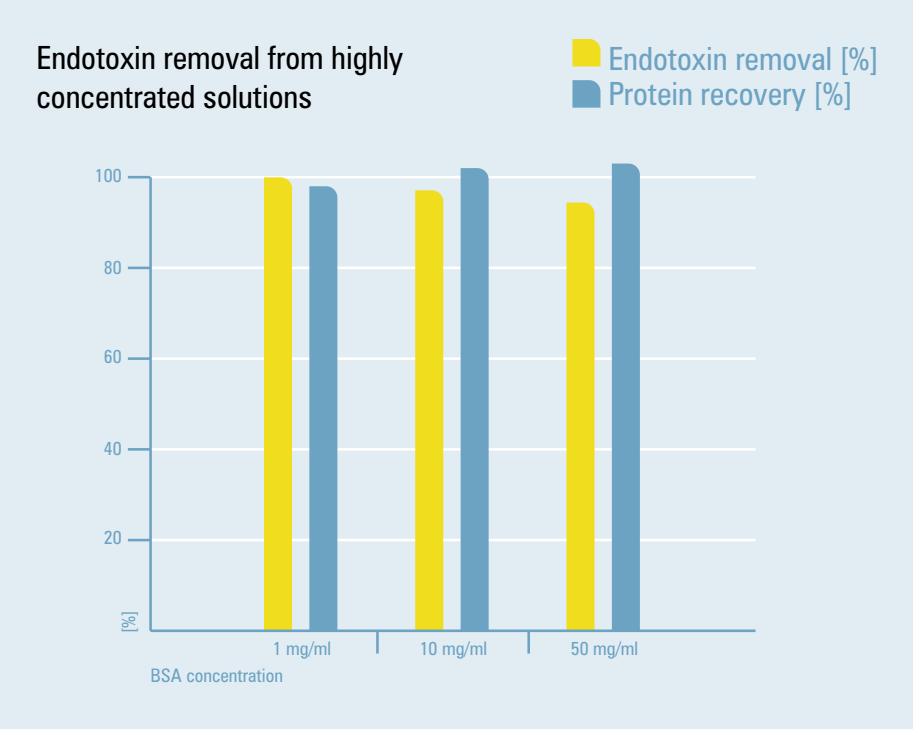


Figure 10: EndoTrap® performance is independent of the protein concentration
BSA solutions spiked with LPS were adjusted to 1 mg/ml, 10 mg/ml, and 50 mg/ml, respectively, and passed through a 1 ml EndoTrap® column. Nearly no difference in endotoxin removal efficiency (yellow bars) and protein recovery (blue bars) can be detected between protein concentrations of 1 mg/ml and 50 mg/ml, respectively.

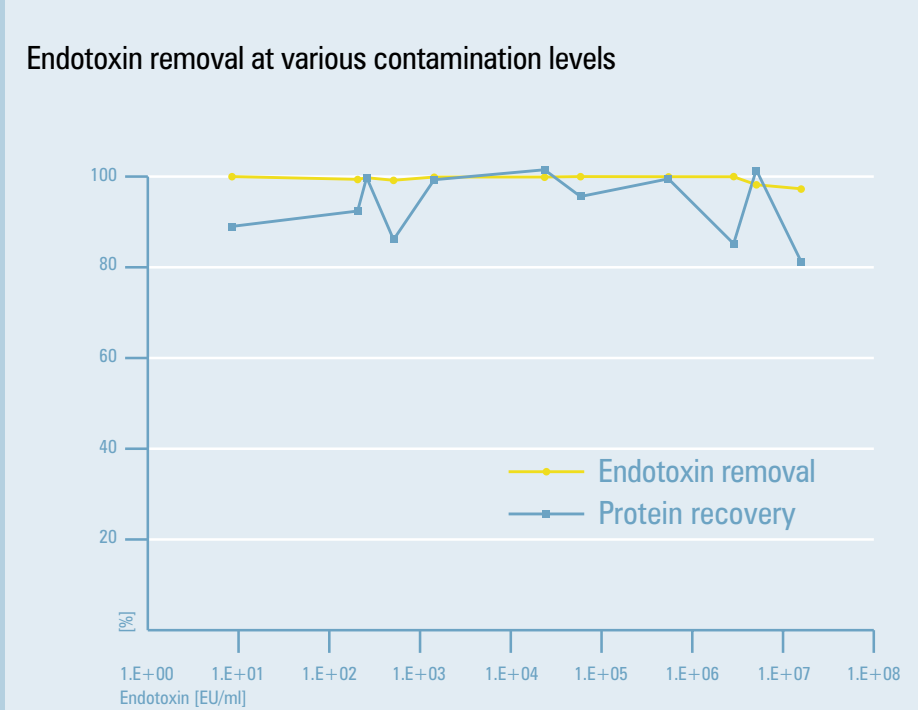


Figure 11: Efficient endotoxin removal at all contamination levels
A BSA solution (1 mg/ml) was spiked with endotoxin concentrations varying from 8.1 EU/mg to 1.6 x 10^7 EU/mg. Endotoxin removal efficiency averaged 99% at all endotoxin levels. Protein recovery averaged 94% over the entire endotoxin range.

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product information

profos

Technical data and specifications

Ligand	EndoTrap®
Mean particle size	90 µm
Bead structure	Highly cross-linked 4% agarose, spherical
Binding capacity	approx. 2 x 10^6 EU/ml
Max. flow rate	4 ml/min
Recommended flow rate	0.5 -1 ml/min
pH stability	
Regular usage	4-9
Cleaning	3-10
Temperature stability	
Regular usage	4°C to room temperature
Storage	4°C to 8°C
Storage buffer	20% ethanol, or regeneration buffer RB + 0.05% NaN_3

Order Info

Product	Contents	Cat. No.
EndoTrap® 5/1	5 x 1 ml columns, ready to use, equilibration buffer, regeneration buffer	311063
EndoTrap® 10	20 ml resin (50%), equilibration buffer, regeneration buffer	311064
EndoTrap® 50	100 ml resin (50%), equilibration buffer, regeneration buffer	311075
EndoTrap® 100	200 ml resin (50%), equilibration buffer, regeneration buffer	311065
EndoTrap® C	column resin, bulk size available	311066
Equilibration buffer	125 ml	311108
Regeneration buffer	125 ml	311067
Empty columns	0.2 ml to 10 ml columns	311068

EndoTrap®'s superior endotoxin binding and removal properties permit application under almost any precondition. EndoTrap® can be used both as benchtop column or batch material, and can also be included in any automated chromatography unit for hands-off purification and polishing steps.

Figure 12: EndoTrap® kit samples
EndoTrap® is available both as pre-poured, ready to use columns for benchtop experiments (EndoTrap® 5/1 [A]), and as bulk resin for batch or chromatography application (EndoTrap® 10/50/100/bulk [B])

For technical questions, order or pricing information please contact:

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