EndoTrap® blue FAQ´s

(Frequently Asked Questions) V 3 – 12/2004

Endotoxin removal system

EndoTrap® blue (**formerly simply EndoTrap® called**) is extended with EndoTrap® red to the EndoTrap®-family in order to increase the application possibilities (larger range of buffers) for your experiments.

Do you have a question with regards to?

Notice: This document contains bookmarks.

EndoTrap® blue in general

What is EndoTrap® blue?

EndoTrap® is an affinity matrix for the efficient removal of bacterial endotoxins from solutions. EndoTrap® can be used in either batch or chromatography mode. EndoTrap® has been developed for the removal of endotoxins from aqueous solutions containing (low or) high molecular weight substances. Frequently, the removal of endotoxins from protein solutions using standard methods (including ultra-filtration, ion exchange chromatography, or two phase extraction) is insufficient.

We refined EndoTrap® to the EndoTrap®-family, so that you can choose a specialized product for your desired applications. EndoTrap® blue and EndoTrap® red have similar characteristics to remove endotoxin. The products are different basically in their buffer compatibilities (you find more detailed information on page 4).

The high affinity of EndoTrap blue ligand to endotoxin enables the efficient capturing of endotoxins, even at very low endotoxin concentrations. The EndoTrap blue ligand is immobilized covalently on beaded agarose to ensure negligible leakage of EndoTrap blue ligand. The endotoxin binding capacity of EndoTrap blue in aqueous buffers is about 2×10^6 EU/ml matrix. Non-specific binding of proteins to EndoTrap blue is extremely low, delivering a mass yield which typically exceeds 95%. The EndoTrap blue system can be reused three times without any loss of endotoxin removal efficiency. In this way it greatly assists you in avoiding artefacts and misinterpretation caused by endotoxin contamination when performing your highly sensitive stimulation experiments in cell culture or animal models.

Customer application and EndoTrap® blue advantages in brief

What are the customer applications in brief?

Customer applications R&D

Cell Culture

- immune modulation e.g. T cell or B cell stimulation
- immune suppression e.g. dendritic cells
- apoptosis e.g. primary endothelial cells
- pro-inflammatory responses
- TLR (toll-like receptors)

Animal Models

- proliferation assays
- vaccine e.g. injection into mouse and monkey
- immune stimulation e.g. HIV envelope
- sepsis

Research Topics

Asthma, Alzheimer's, Airway hyper responsiveness, Cancer, crop pharmaceuticals, Diabetes, Rheumatism, Atherosclerosis, Cystic fibrosis; Sepsis, fever – patient shock, SIRS – systemic inflammatory response syndrome, Multiple sclerosis, Crohn´s disease, Diarrhoea, Drug discovery

Which types of substances and in which concentration can I apply to the EndoTrap® blue column?

In general, every type of substance can be applied onto the column, as long as it can pass through the column. There is no limit concerning the MW of proteins or substances.

Substances like proteins, peptides, antibodies or plasmid DNA are possible.

Protein concentrations of above 50 mg/ml have successfully been applied onto the system. However we recommend a work concentration of 1-10 mg/ml.

EndoTrap blue removes endotoxin from proteins with isoelectric points (pI) from 5 to 9.

EndoTrap blue works also with DNA (tested for a plasmid DNA).

What are the key advantages of EndoTrap® blue in brief?

- Reliable endotoxin removal from protein solutions, > 99% per round, 2 x 10^6 EU binding capacity
- Highest and best specificity of any current endotoxin removal system
- Highest protein recovery rate: average of 95%
- Fast and easy Flow-Through System, no incubation needed
- Wide pH range, Ionic strength
- Easily regenerated
- Applicable for cell cultures, solutions of proteins, peptides, antibodies, plasmid DNA, and pharmacological components.

What are the specifications of EndoTrap® blue?

Column dimensions	1 ml	
Ligand	EndoTrap blue	
Binding capacity	2.000.000 EU/ml resin	
pH stability	pH 4 - 9	
Support matrix	Highly cross-linked 4% agarose, spherical beads	
Void volume	0.3 to 0.5 ml	
Mean particle size	90 μm	
Storage	At 4 °C in regeneration buffer (RB) supplemented with 0.02% sodium azide or in 20% ethanol	
Max. flow rate	1 ml/min	
pH stability	Regular use	4 - 9
	Cleaning	3 - 10
Temperature stability	Regular use in range between 4 °C and room temp.	
	Storage	4 °C to 8 °C
Shelf live	12 months	

What is the basis of the EndoTrap® blue system?

System is based on proteineous affinity ligand derived from a bacteriophage, which binds to endotoxins with a high affinity constant. EndoTrap blue is not an antibody.

What the different characteristics between EndoTrap® blue and EndoTrap® red?

EndoTrap blue has similar characteristics as EndoTrap red to remove endotoxin. The products are different basically in their buffer compatibilities. One different is, that the equilibration buffer for EndoTrap blue needs additives from calcium (kit included equilibration buffer is ready to use and contains therefore 50 μM Ca^{2+}) and is therefore not suitable for PBS or Calcium chelators (e.g. EDTA) containing buffers. The protein recovery rate of EndoTrap blue is easily better in direct comparison to EndoTrap red. Nonetheless normally both EndoTrap endotoxin removal systems have a protein recovery rate of over 90%!

Following tables give a short overview of the differences of both EndoTrap® systems.

The most important differences between both EndoTrap®-family products - … to choose the right product for your desired application:

	EndoTrap® blue	EndoTrap® red
▪ Customer specific equilibration buffer have to enriched with calcium	Yes	**No**
▪ PBS can be used as equilibration buffer	only when enriched freshly with 50-100 μM Ca^{2+}!*	**Yes**
▪ suitable with EDTA, and other Calcium chelators containing buffers	No	**Yes**
▪ Ionic strength	up to 600 mM NaCl	up to 250 mM NaCl
▪ pH (buffer)	pH 4-9	pH 6-9
▪ endotoxin starting contamination well under 10 EU/ml	+	+++

* Further information for the preparation of buffers you find here on page 9 "proteins, medium and buffers".

Comparison of the specifications of EndoTrap® blue and EndoTrap® red

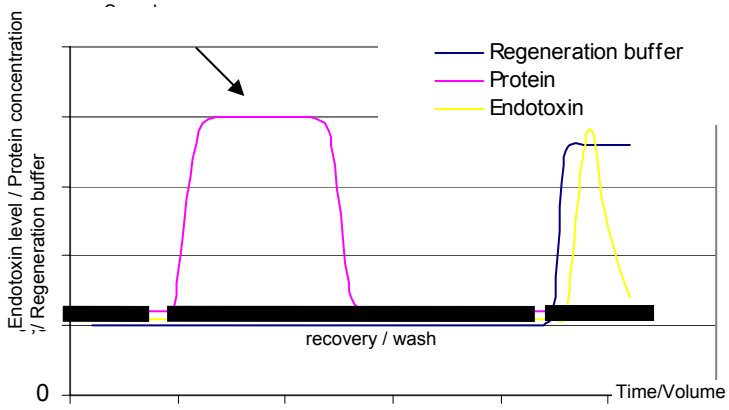
	EndoTrap® blue	EndoTrap® red
Regeneration buffer (included)	"**HEPES-buffer**", pH 7.5 (endotoxin concentration < 0.02 EU/ml)	"**PBS-buffer**", pH7.4 (endotoxin concentration < 0.02 EU/ml)
Equilibration buffer (included)	"**HEPES-buffer**", pH 7.5 enriched with 0.1 mM $CaCl_2$,) (endotoxin concentration < 0.02 EU/ml)	"**PBS-buffer**", pH 7.4 (endotoxin concentration < 0.02 EU/ml)
If you want to use your own buffer for equilibration (instead of the kit included equilibration buffer)	We tested EndoTrap blue successful with BORAT, TRIS, MOPS, MES, PIPES (binding of EndoTrap blue to LPS depends on Calcium, therefore you have to add 50-100 μM Ca^{2+} to your buffers!)	We tested EndoTrap red successful with HEPES, BORATE, TRIS, MOPS, MES, PIPES, Citrate-, Acetate-, Glycine- and Carbonate-buffers
Tested type of substance which can be applied onto the column	▪ proteins ▪ peptides ▪ antibodies ▪ plasmid DNA	▪ proteins ▪ peptides ▪ antibodies
pI of applied proteins	pI from 5-9	pI from 5-9
pH (buffer)	pH 4-9	pH 6-9
Ionic strength	up to 600 mM NaCl	up to 250 mM NaCl
Chaotropic substances	up to 2 M urea (pH 7)	not tested
Recommend working concentration of applied substances	1-10 mg/ml	1-10 mg/ml
Recommend sample volume	up to 50 ml	up to 50 ml
Tested substances which **interfere** with the performance of Endo-Trap and have therefore an inhibitory effect for the binding to LPS	▪ 10 mM NaOH ▪ 2 M urea at neutral pH ▪ SDS and other detergents ▪ Citrate ▪ ETDA, and other Calcium chelators (EGTA, HEDTA, NTA)	▪ SDS and other detergents
Tested substances which **do not interfere** with the performance of EndoTrap	up to 10 mM DTT (Dithiothreitol)	not tested
Tested kinds of LPS (bacteria strain) [for detail information please read our FAQ´s]	▪ Escherichia coli, K12, R1, R2, R3, R4 ▪ Salmonella enterica ▪ Citrobacter freundii ▪ Citrobacter amalonaticus ▪ Citrobacter koseri ▪ Pseudomonas aeruginosa ▪ Pseudomonas stutzeri ▪ Enterobacter aerogenes ▪ Enterobacter asburiae ▪ Enterobacter cloacae ▪ Aeromonas hydrophilia	▪ Escherichia coli, K12 ▪ Salmonella enterica ▪ Citrobacter freundii ▪ Pseudomonas aeruginosa *for* ▪ Klebsiella pneumoniae ▪ Serratia marcescens *we recommend EndoTrap red!*

EndoTrap® blue performance

How can I use EndoTrap® blue?

EndoTrap blue can be used either in batch or column mode. In general, endotoxin removal of high endotoxin levels is more practical in the column mode. Batch mode may be used for small volumes or to increase contact time. However, parameters such as pH, ionic strength, temperature, contact time, etc. **might have to be optimized for each application** to obtain maximum endotoxin removal with minimum loss of product.

What does a typical chromatogram using EndoTrap® blue endotoxin removal system look like?



Can EndoTrap® blue be used at 4 °C?

Yes, EndoTrap blue properties are the same at 4 °C as at room temperature.

Is it possible to use EndoTrap® blue with chaotropic substances, like urea?

Yes, up to 2 M urea can be used at pH 7 without any significant decrease of endotoxin removal efficiency.

Are substances known which can interfere with the performance of EndoTrap® blue?

Yes. The following substances interfere with EndoTrap blue performance:

- More than 10 mM NaOH.
- More than 2 M urea at neutral pH
- SDS and other detergents.
- EDTA, and other Calcium chelators (EGTA, HEDTA, NTA) should also be avoided.

Do reducing agents like Dithiothreitol (DTT) disturb the endotoxin removal of EndoTrap® blue?

No. EndoTrap blue can be used with DTT (up to 10 mM) or pre-washed with DTT before use.

Is it possible to use EndoTrap® blue at high ionic strengths?

Yes. Up to 600 mM NaCl EndoTrap blue can be used without any significant loss of endotoxin removal efficiency.

Can the EndoTrap® blue material be autoclaved, sanitized or otherwise sterilized?

EndoTrap blue can not be autoclaved, but you can wash the column in 30% Ethanol.

There is, however, the drawback of sanitation with sodium hydroxide. This would destroy the ligand. Sanitation with NaOH is therefore not recommended!

However, the ligand is rather stable against Urea till 2 M Urea at neutral pH does not harm the ligand.

Can the EndoTrap® blue material be regenerated?

Yes, the functional regeneration of the column will be made with the provided buffer. The EndoTrap blue system can be reused three times without any loss of endotoxin removal efficiency.

As storage buffers we recommend our provided regeneration buffer supplemented with 0.02% sodium azide. Regenerated EndoTrap blue matrix should be stored at 4 °C, do not freeze.

How can I increase the efficiency of endotoxin removal?

To reach greater efficiency, make the contact time as long as possible. Flow rates below 0.2 ml/min can improve the performance in tricky situations. If the sample volume is very small (i.e. in the range of the void volume of 0.3-0.5 ml), you can incubate the sample on the column for 30 minutes prior to elution (stop liquid flow by capping the bottom and then the top of the column).

If you want to use your own buffer, add at least 50 μM calcium (we recommend $CaCl_2$) only freshly into your buffer just before you will use it for the polishing of your solution.

Which final endotoxin level can be achieved with EndoTrap® blue?

With repetitive use of EndoTrap blue (buffer), you can go down to 0.005 EU/ml.

With repetitive use of EndoTrap blue (protein), you can go down to 0.1 EU/ml. Then the efficiency of the EndoTrap blue system as competitive systems decreases with low endotoxin contamination levels. At 0.1 EU/ml the removal efficiency is approx. 70%.

What is the main advantage of EndoTrap® blue over other products?

EndoTrap blue has the highest protein recovery rate and a great endotoxin removal rate with an easy to use flow-through system. It also has a very stable performance and a wide variety of conditions. (pH and ionic strength [salt]).

Does the EndoTrap® blue system bind all pyrogens?

No, since the class of pyrogens consists of all kind of molecules and polymers with stimulating activity. But the vast majority comes from bacterial endotoxins. EndoTrap blue binds to the conserved region of the inner core of the lipopolysaccharide (LPS) molecule and can thereby bind to all kinds of endotoxin species.

Tested species are: Escherichia coli O55:B5, Escherichia coli K12W3110, Pseudomonas aeruginosa rough and smooth mutant, Pseudomonas stutzeri, Citrobacter freundii, Citrobacter koseri, Enterobacter aerogenes, Enterobacter asburiae, Enterobacter cloacae, Aeromonas hydrophilia. Their lipopolysaccharides bind with an efficiency of 98-99.9% to the EndoTrap blue ligand.

Does the EndoTrap® blue system bind yeast pyrogens?

No, because the yeast cell wall is completely different from the gram negative bacterial outer membrane. But certain bacterial species can occur in laboratory water supplies and are common sources for endotoxin contamination during downstream processes. LPS from most of these water contaminants is efficiently recognized and removed by EndoTrap blue. (See above).

My Endotoxin levels are very high after usage of EndoTrap® blue. What can be the reason for it?

- LAL-assay is prone to errors.
- EndoTrap blue needs some Ca^{2+} (about 50 – 100 μM) in solution. Are there any calcium chelators in the used buffer?
- Contaminated buffers – please check all buffers for ET contamination.
- Endotoxin contaminants on the EndoTrap blue column: please do not forget to wash the EndoTrap blue column with buffer before applying the sample. Test that wash fraction for endotoxin.

  Endotoxin levels of supplied materials have been tested to be below detection limits. Equilibration, regeneration buffers and the flow path of the EndoTrap blue column are tested for the presence of endotoxins. Limit: endotoxin concentration $<$ 0.02 EU/ml.
- PBS buffer – traces of Ca^{2+} are necessary for efficient binding of EndoTrap blue. Phosphate and Ca^{2+} form an insoluble complex and will precipitate.

  When you can **only** use PBS buffer we recommend the following procedure:

  Your PBS buffer must contain Ca^{2+} between 50 and 100 μM. The Ca^{2+} must be **freshly** added to the buffer. That means that you add Ca^{2+} (we recommend $CaCl_2$) only directly into your PBS buffer before you will use it for the polishing of your solution.

Which precautions should I take when I use EndoTrap® blue?

- ! **All used materials such as containers or pipette-tips and buffers must be endotoxin free.** Glassware is preferred, as endotoxins can be removed by heat treatment (200 °C, 4 h; 250 °C, 1 h).
- ! If you used your own buffers: **Buffers used for endotoxin removal with EndoTrap blue must contain at least 50 μM Ca^{2+}!** We recommend adding Ca^{2+} always freshly to your buffers.
- ! Buffers should be prepared from endotoxin free materials and endotoxin free water.
- ! When using EndoTrap blue columns, all buffers including equilibration buffer (EB) and regeneration buffer (RB) should be degassed prior to use. When using EndoTrap blue gel slurry, degas slurry prior to use – see "degassing" (page 7) for details.
- ! Avoid proteases and organic solvents.

Is the performance for all situations the best?

We optimized the product for a broad set of conditions, so you can remove endotoxins from your proteins, peptides, antibodies or plasmid DNA solution at conditions which are best for your proteins, peptides, antibodies or plasmid DNA. (All of profos tested experiments are described in the EndoTrap blue product description!) But due to the nature of any given

proteins, peptides, antibodies or plasmid DNA the performance can vary. E.g. to remove endotoxins from lipo-proteins or membrane proteins is much more difficult in comparison to proteins without hydrophobic patches. If you have a rather unstable protein this protein is more likely to denature on surfaces and form aggregates on (any) column and would therefore be prone to protein loss.

However, parameters such as pH, ionic strength, temperature, contact time, etc. **might have to be optimized for each application** to obtain maximum endotoxin removal with minimum loss of product.

Degassing

Is degassing obligatory necessary for performance?

No. If you don't have a vacuum membrane pump, you can also work with supplied buffers. But be aware of bubbles!

Which procedure is sufficient for degassing?

Place bottles (opened lids!) of RB, EB and/or EndoTrap blue Gel in desiccator, apply vacuum with membrane pump for 1 hour.

Ligand & Leakage

What is the nature of the ligand? Is it a protein?

System is based on proteineous affinity ligand, which binds to endotoxins with an extremely high affinity constant.

What is known about leakage of EndoTrap® blue ligand?

Leaching can be observed by each covalently-based system. The EndoTrap blue ligand is immobilized covalently on beaded agarose to ensure negligible leakage of EndoTrap blue ligand. Therefore leakage is very low, about 10-100 ng Ligand/ml solvent; it is very important to wash the column before use. EndoTrap blue ligand linkage to Sepharose support is extremely stable; leaching is close to the detection limit of 0.004 ppm.

Is the ligand toxic or does the ligand shows any stimulating/activating effect on cell cultures?

No stimulating effect has been reported so far.

EndoTrap blue active ligand was tested for potential immune stimulatory effect on cultured spleenic cells. No effect regarding release of various cytokines has been monitored. Therefore EndoTrap blue suits best for all kind of cell culture based assays.

EndoTrap® blue storage and delivery

How can I store EndoTrap® blue?

EndoTrap blue is supplied as pre-packed columns or as 50% slurry in regeneration buffer with 0.02% sodium azide.

EndoTrap blue is stable for at least 4 weeks between 4 °C and 25 °C. Regenerated EndoTrap blue matrix should be stored at 4 °C in regeneration buffer (RB) supplemented with 0.02% sodium azide. Do not freeze.

Which material is not provided, what do I need in addition?

Storage buffer	please add to the necessary volume of regeneration buffer 0.02% sodium azide
Exsicator	for the degassing of buffers
Holder for column	

Proteins, medium and buffers

Does the sample (proteins, peptides, antibodies, plasmid DNA) concentration have an effect on endotoxin removal efficiency?

Remove all aggregates from your sample solution by centrifugation at 6.000 x g for 30 minutes. Make sure your solution is not too viscous or it will hardly pass through the column. Depending on the nature of your protein, 10 mg/ml work fine. We recommend a protein working concentration of 1-10 mg/ml. By passing your solution through the column and washing with equilibration buffer (EB), your protein solution will be diluted.

Which (proteins, peptides, antibodies, plasmid DNA) buffer systems are compatible with EndoTrap® blue?

Customer-specific buffers may be used for equilibration and endotoxin binding onto the column. Endotoxin removal with EndoTrap blue works effectively in the pH range of 6.5 - 9.0 and in presence of NaCl concentrations in the range of 50 – 600 mM. Buffers like HEPES, TRIS, MOPS, MES, and PIPES may be used. Parameters such as pH, ionic strength, temperature, contact time etc. might **have to be optimized for each application** to obtain maximum endotoxin removal with minimum loss of product. Citrate buffers and chelators of divalent cations (like EDTA) must be avoided.

Do not forget: Your buffer must contain 50-100 μM Ca^{2+}! We recommend adding Ca^{2+} (e.g. $CaCl_2$) always freshly to your customer-specific buffer.

When you can **only** use PBS buffer we recommend following:

Your PBS buffer must contain Ca^{2+} at least 50 μM. The Ca^{2+} must be **freshly added** to the buffer. That means that you add Ca^{2+} (we recommend $CaCl_2$) only directly into your PBS buffer before you will used it for the polishing of your solution.

Would it also work for membrane proteins?

In general, it would work with membrane proteins, but detergents would inhibit the performance of EndoTrap blue.

Is it possible to use EndoTrap® blue with chaotropic substances, like urea?

Yes, up to 2 M urea can be used at pH 7 without any significant decrease of endotoxin removal efficiency.

Do reducing agents like Dithiothreitol (DTT) disturb the endotoxin removal of EndoTrap® blue?

No. EndoTrap blue can be used with DTT (up to 10 mM) or pre-washed with DTT before use.

I already have a low Endotoxin concentration, is it worth applying EndoTrap® blue?

Typically lowest possible EU start concentration in protein solutions is: 1-10 EU/ml (yielding 80% removal rate) results in 0,1 EU/ml (lowest practical level).

Can I contaminate my sample (proteins, peptides, antibodies, plasmid DNA) solution with endotoxins by using EndoTrap® blue?

All materials and solutions supplied with the EndoTrap blue Kit are tested. We guarantee endotoxin levels significantly below 0.02 EU/ml (See also certificate of analysis).

I lost all my (stable) protein on the column. What should I do?

This is a very unusual situation. We will assist you in the trouble shooting. First, we will need some more details on your protein, your buffer system, how you used EndoTrap blue and which measurements have been done. Then we will discuss with the product manager and the research team to trace down the reason for this loss.

EndoTrap® blue application - the column mode

What is Column mode? What is the protocol for it?

Chromatography is traditionally made in two modes: batch (or discontinuous) and continuous (column mode) chromatography. EndoTrap blue can be used in either a batch mode or a chromatography column. Columns are easily prepared by packing with the depyrogenated EndoTrap blue resin as 50% slurry in sterile buffer. Column operation is generally more effective than batch processing.

What does the EndoTrap® blue prepacked columns consist of?

prepacked columns

EndoTrap® blue 1/1	EndoTrap® blue 5/1
1 x 1 ml column, 125 ml Equilibration Buffer[1], 125 ml Regeneration Buffer	5 x 1 ml column, 250 ml Equilibration Buffer[1], 125 ml Regeneration Buffer

Preparation

1. To use a pre-packed column, remove the top cap first. This prevents bubbles from being drawn into the gel. Next, remove button cap and place the column in a suitable holder. Allow storage solution to drain completely from column.
2. If you use EndoTrap blue gel slurry, fill the slurry in an appropriately sized column and allow gel to settle for 30 minutes.

Activation and Endotoxin Removal

1. Wash column with 6 column volumes of regeneration buffer (RB).
2. Equilibrate the column with 6 column volumes of equilibration buffer (EB)[1].
3. Apply sample onto the column, start collecting immediately. Column can be filled up to the edge of the column (appr.: 4 ml for the small columns). The column can be constantly filled up, until the whole sample is completely filled in. Afterwards let sample drain completely from column.
4. Add 6 volumes of equilibration buffer (EB). Collect all. A fraction size of 1 ml is recommended.

Regeneration and Storage

5. If you want to store the column, allow the equilibration buffer (EB) to drain completely from column. Apply 1 ml of regeneration buffer (RB) supplemented with 0.02% sodium azide and store at 4 °C. EndoTrap blue is stable as far as the stated expiry date when correctly stored.
6. Prior to the next run make sure you start with step 1 of "Activation and Endotoxin removal".

[1] **Equilibration buffer „blue" (EB):** 20 mM Hepes, 150 mM NaCl, 0.1 mM $CaCl_2$, pH 7.5; endotoxin concentration < 0.02 EU/ml

Performance characteristics of the column mode

Is it possible to use a peristaltic pump instead of gravity flow?

Yes, but do not exceed the speed over 1 ml/min. The slower the speed, the more efficient the endotoxin removal is. Gravity flow guarantees a flow rate of about 0.5 ml/min. Make sure that all tubing and fittings are absolutely endotoxin free!

When does the sample (proteins, peptides, antibodies, plasmid DNA) elute?

The void volume of 1 ml EndoTrap blue Gel is 0.3-0.5 ml, the sample begins to emerge immediately after that volume has passed through the column.

Can I reuse the column?

Yes, you can. Make sure you follow the protocol exactly. The EndoTrap blue system can be reused three times without any loss of endotoxin removal efficiency.

If you run the same sample several times over the same column (after reactivation!) you will continuously decrease the level of endotoxins. Remember that the sample (proteins, peptides, antibodies, plasmid DNA) concentration will slightly decrease with each round.

If you want to decrease the endotoxin level below 1 EU/ml we recommend slowest flow rates or batch mode to increase contact time. Hereby you obtain however a smaller protein (peptides, antibodies, plasmid DNA) recovery rate.

To avoid cross contamination of samples (proteins, peptides, antibodies, plasmid DNA), we highly recommend using one column for one sample solution.

Which sample volume can be applied onto 1 ml column?

1 to 10 column volumes usually work best. Up to 50 ml can be applied onto a 1 ml EndoTrap blue column without loss of endotoxin removal efficiency.

Which inhibitory compounds for EndoTrap® blue are known?

EDTA and other calcium-chelators as EGTA, HEDTA, NTA, Citrate, SDS and other detergents.

Can EndoTrap® blue also be performed on fully automated liquid chromatography system?

No, EndoTrap blue 5/1 is a gravity flow system, the EndoTrap blue column can not be used for liquid chromatography systems directly. But if you want to use liquid chromatography systems, you can use the EndoTrap blue columns, if you arrange yourself suitable adaptors. Be care, that the run time is not be higher than 1 ml/min so that the gel bed does not compress.
It is also possible that you fill the EndoTrap blue resin in a pour liquid chromatography column. (The empty liquid chromatography column can not be bought from profos!)

How often can I regenerate EndoTrap® blue columns or column material?

You can regenerate the columns three times without significant loss of endotoxin removal efficiency.

I lost all my (stable) protein on the column. What should I do?

This is a very unusual situation. We will assist you in trouble shooting. First, we will need some more details on your protein, your buffer system, how you used EndoTrap blue and which measurements have been done. Then we will discuss with the product manager and the research team to trace down the reason for this loss.

EndoTrap® blue application - the batch mode

What is Batch Mode? What's the protocol for it?

Chromatography is traditionally made in two modes: batch (or discontinuous) and continuous (column mode) chromatography. EndoTrap blue can be used in either a batch mode or a chromatography column. Column operation is generally more effective than batch processing. For batch depyrogenation, the settled gel is simply added directly to the sample solution. Several contact times ranging from 3 to 20 min should be tested to determine the most complete removal of endotoxin.

What does the EndoTrap® blue resin kits consists of?

resin (50% slurry)

EndoTrap® blue 10	EndoTrap® blue 50	EndoTrap® blue 100
20 ml resin (50% slurry), 250 ml Equilibration buffer[1], 250 ml Regeneration buffer	100 ml resin (50% slurry), 125 ml 10x Equilibration buffer[1] 125 ml 10x Regeneration buffer	200 ml resin (50% slurry),, 250 ml 10x Equilibration buffer[1] 250 ml 10x Regeneration buffer

All centrifugation steps should be carried out at 1.200 x g for 2 min at room temperature! Several contact times ranging from 3 to 20 min. should be tested to determine the most complete removal of endotoxin. We recommended using a relationship between sample volume and resin volume of at least 10:1 (e.g. 10 ml of your protein sample to 1 ml EndoTrap blue resin).

Preparation

1. Remove storage buffer from resin by centrifugation and aspirate the supernatant.

Activation and Endotoxin removal

2. Add 2 resin volumes of regeneration buffer (RB) to 1 volume of EndoTrap blue resin, mix by gently shaking the tube for 5 sec.; centrifuge, and aspirate the supernatant. Repeat this step 2 times.
3. Add 2 resin volumes of equilibration buffer (EB)[1], mix by gently shaking the tube for 5 sec.; centrifuge and aspirate the supernatant. Repeat this step 2 times.
4. Add the sample (i.e. 10 ml of your protein sample to 1 ml EndoTrap blue resin) and incubate for at least 30 min at RT. Gently rock or rotate the tube while incubating.
5. Centrifuge at 1.200 x g for 5 min and transfer the supernatant to an endotoxin free tube.

Regeneration and Storage

6. EndoTrap blue resin can be stored at 4°C in regeneration buffer (RB) supplemented with 0.02% sodium azide.
7. Prior to the next run make sure you start with step 1 of "**Activation and Endotoxin removal**".

[1] **Equilibration buffer „blue" (EB):** 20 mM Hepes, 150 mM NaCl, 0.1 mM $CaCl_2$, pH 7.5; endotoxin concentration < 0.02 EU/ml

EndoTrap® blue manufacturing and quality

How do you ensure quality?

We generate a certificate of analysis (COA) where following parameters are checked:

Each batch

- Endotoxin Removal:
  - Test with BSA (1 mg/ml, 1000 EU/ml)
  - Endotoxin removal rate has to be above 98%
  - Protein recovery with BSA as standard protein has to be > 90%
- Endotoxin levels of supplied materials have to be below detection limits. Equilibration and Regeneration buffers are tested for the presence of endotoxins. Limit: endotoxin concentration < 0.02 EU/ml.
- Capacity test: minimal capacity is 2×10^6 EU per 1 ml EndoTrap blue

Is your manufacturing facility certified to any cGMP or equivalent standards?

The manufacturing process is not currently certified in cGMP standards, but SOPs and strict quality control guarantee optimal products.

Do you have a drug master file or regulatory support file?

No, but we are currently developing a regulatory support file.

EndoTrap® blue references

Do you have references or publications?

References:

- Paul–Ehrlich-Institut; Dr. Scheurer; Abteilung Allergologie 5/4; Paul-Ehrlich-Str. 51-59, 63225 Langen, Germany, Tel. +49 (0) 6103 77 2260.
- Robert-Koch-Institut; Dr. Denner; Abteilung P23; Nordufer 20; 13353 Berlin; Germany, Tel. +49 (0)1888 754-2800.

Please visit www.profos.de and look for our latest press releases.

Publication:

- J Biol Chem. 2004 Nov 12;279(46):47906-11. **Lipopolysaccharide-free Heat Shock Protein 60 Activates T Cells.** Osterloh A, Meier-Stiegen F, Veit A, Fleischer B, von Bonin A, Breloer M. Bernhard-Nocht-Institute for Tropical Medicine, 20359 Hamburg, Germany.

Profos Service: Endotoxin removal and endotoxin detection

What do you charge for Endotoxin removal service?

There is no standard pricing system: it depends on the project. We provide you gladly an offer on request.

Does Profos offer a LAL assay?

We do offer a service for measurement of your endotoxin levels using a quantitative, kinetic chromogenic LAL assay. The price strongly depends on number of samples. Please inquire.

Guidelines for sample submission for Endotoxin Detection Service

The customer delivers samples either in aqueous solution or in solid state. If you send aqueous solution, volume of the sample should be at least 100 µl. Please indicate buffer composition, pH in our questionnaire. If sample is a protein sample, please also indicate, if known, the concentration of the sample.
For details concerning the samples, please fill in the above mentioned questionnaire. We like to send you the questionnaire on request. Be aware that some components may affect LAL test (e.g. SDS, EDTA).

Required information's about the sample:

- Buffer composition (disturbing substances for LAL-Test: EDTA, detergents, proteases, high concentrations of salt, proteins or other substances like lipids and sugars)
- Appropriate buffers: Tris, Hepes, PBS
- pH-range should be about 6-8
- Estimated endotoxin content of the sample (e.g. Does the sample derives from gram negative bacteria? Or from cell culture?)
- Does the sample contain glucans? (Does the sample derive from yeast cultures?)

Informations for LAL-Test

Quantitative, kinetic chromogenic LAL assay.

If sample contains disturbing components, the sample is diluted in an appropriated buffer.

We use a standard curve with following LPS concentrations:
0.005 EU/ml, 0.05 EU/ml, 0.5 EU/ml, 5 EU/ml, 50 EU/ml (in endotoxin free water)

Negative control:	endotoxin free water
Positive control:	sample spiked with 0.5 EU/ml (recovery rate has to be in the range of 50-200%)

Preliminary screening determines the concentration of the sample that allows for compatibility with the LAL test. Three dilutions of the sample are carried out to ensure that measured values of the sample are in the range of the standard curve.

Information, pricing and distribution

What other recommendations do you have?

Pay attention to the precautions section.
Or contact our service team by phone (+49 941 942 62 0) or email (inquiry@profos.de).

How much does EndoTrap® cost?

For further pricing information please request for a price-list.

Do you have distributors?

Yes, we do have distributors, but there is also a convenient way to order directly from us. We also sell directly in a very fast and easy way. For more information please inquire.

Could you send me some information about EndoTrap® blue?

EndoTrap blue information booklet, 10 pages in depth information, package insert and current price-list are available on request.

How can I order EndoTrap® blue or contact Profos for further questions?

By phone: + 49 (0) 941 942 62 0
By fax: + 49 (0) 941 942 62 20
By email: inquiry@profos.de
By post: profos ag
Josef-Engert-Str. 9
D - 93053 Regensburg

Profos in general

What does Profos do and what does "Profos" stand for?

Profos utilizes bacteria's natural enemies (bacteriophages) to design new and innovative tools for the biotech, food and feed industries, e.g. concerning the highly sensitive detection of bacteria or the powerful endotoxin removal. Profos has a wide expertise in protein science, an extended know-how in bacteriophage technology, and relies on the broadest platform in phage ligand technology, covered by 11 granted and pending patent families. These skills we want to use for products, which are faster and more effective than any previous – all that, moreover, at a better value.

Profos was established in year 2000 as a spin-off from the University of Regensburg and is currently employing 22 people. Profos stands for **p**hage p**ro**tein **fo**lding and **s**tabilization.

For further company information, visit Profos on the Internet at www.profos.de.