

FAQs EndoTrap®

Endotoxin removal system – Chromatography system for column or batch mode

Introduction

EndoTrap® is an affinity chromatography resin for the efficient removal of bacterial endotoxins (lipopolysaccharides/LPS) from aqueous solutions containing low or high molecular weight substances such as proteins and nucleic acids. EndoTrap® can be employed both in column or batch mode, by gravity flow or on fully automated liquid chromatography systems.

Frequently, endotoxin removal from protein solutions is insufficient with standard methods e.g. ultra filtration, ion exchange chromatography, or two phase extraction. This is why we invented EndoTrap®.

The EndoTrap® family has three members:

EndoTrap® blue

with a broad pH and salt tolerance, works best with calcium or magnesium compatible buffers such as TRIS, HEPES, MOPS but also PBS, if enriched freshly with 0.1 mM Ca/Mg²⁺.

EndoTrap® red

especially for the use with PBS based samples.

EndoTrap® HD

is a further development of EndoTrap EndoTrap® blue for challenging samples and large scale endotoxin removal in e.g. biopharmaceutical production processes. For EndoTrap® HD additionally are available a Regulatory Support File (RSF) and the EndoTrap® leakage ELISA.

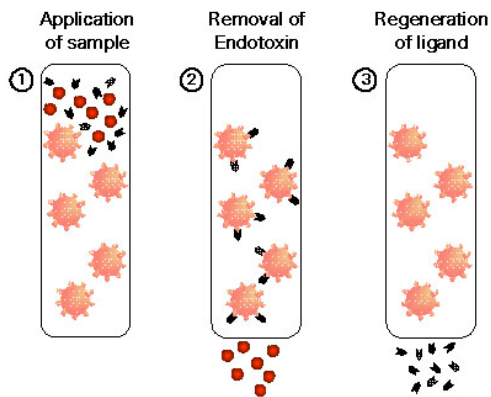
This multipurpose product portfolio covers a broad range of conditions with no special equipment or buffer requirements. The EndoTrap® blue/red kits include all buffers needed. EndoTrap® HD is supplied including instructions for buffer preparation and can be supplied including buffers on request.

The following tables give a short overview on the differences of the EndoTrap® systems. For detailed information about EndoTrap® blue, EndoTrap® red and EndoTrap® HD please read our FAQs or inquire the package inserts at www.hyglos.de.

Specifications of the EndoTrap®-family

Ligand	Protein ligand (bacteriophage derived)
Binding capacity	EndoTrap blue / red : 2.000.000 EU/ml resin (each cleaning step theoretically yields a two log reduction of LPS) EndoTrap HD: 5.000.000 EU/ml resin (each cleaning step theoretically yields a three log reduction of LPS)
Support matrix	EndoTrap blue/red: Highly cross-linked 4% sepharose, spherical beads EndoTrap HD: hydrophilic, cross-linked metacrylic polymer
Void volume	0.3 to 0.5 ml / ml resin
Mean particle size	40 - 90 µm
Max. flow rate	EndoTrap blue/red: 0.2 - 1 ml/min (gravity flow) EndoTrap HD: up to 100 ml/h/ml resin
Max. pressure	EndoTrap blue/red: 3 bar, 0.3 MPa, EndoTrap HD: 5.5 bar, 0.55 MPa (when using automated systems)
Storage	At 2 - 8 °C in regeneration buffer supplemented with 0.02% sodium azide or 20 % ethanol. Do not freeze!
Shelf life	EndoTrap® is stable until the stated expiry date when stored correctly at least 2 years from production date.

Principle



Principle of EndoTrap®:

1. Endotoxin-contaminated proteins and aqueous solutions are applied
2. Endotoxin is captured, proteins elute
3. Regeneration of ligand by using regeneration buffer

Products of the EndoTrap® - family

Product	Contents	EndoTrap® blue Cat. No.	EndoTrap® red Cat. No.
EndoTrap 1/1	1 x 1-ml column, ready to use, equilibration buffer, regeneration buffer	311053	321053
EndoTrap 5/1	5 x 1-ml columns, ready to use, equilibration buffer, regeneration buffer	311063	321063
EndoTrap 10	10 ml settled resin, supplied as 50% slurry in regeneration buffer; equilibration buffer, regeneration buffer	311064	321064

Product	Contents	EndoTrap® blue Cat. No.	EndoTrap® red Cat. No.
EndoTrap 50	50 ml settled resin, supplied as 50% slurry in regeneration buffer; equilibration buffer, regeneration buffer	311075	321075

Product	Contents	EndoTrap® HD Cat. No.
EndoTrap 10	10 ml settled resin, supplied as 50% slurry in regeneration buffer	800034
EndoTrap 50	50 ml settled resin, supplied as 50% slurry in regeneration buffer	800035
EndoTrap 250	250 ml settled resin, supplied as 50% slurry in regeneration buffer	800036
EndoTrap Bulk	Bulk resin supplied as 50% slurry in regeneration buffer	800037
EndoTrap Leakage ELISA for EndoTrap blue/ EndoTrap HD	12 x 8 well stripes 1 vial POD-antibody 1 vial Standard 20 ml ABTS substrate, ready to use	800033

Storage

EndoTrap® is supplied as **prepacked columns** (EndoTrap® 1/1 or 5/1, *1-ml column material*) or as **50% slurry** (EndoTrap® 10, 50 or 250, *resin material*) in regeneration buffer (RB) supplemented with 0.02% sodium azide. EndoTrap® is stable until the stated expiry date when stored correctly. Regenerated EndoTrap® matrix should be stored at 2-8°C in regeneration buffer (RB) supplemented with 0.02% sodium azide.

You can also use 20% ethanol as storage buffer; however, the storage time will be only 4 weeks then.

Do not freeze!

EndoTrap® FAQs

What is EndoTrap®?

It is an affinity chromatography resin based on a protein from bacteriophages, which binds to endotoxins with high efficiency. EndoTrap® binds to the conserved region of the inner core of the LPS molecule thus it is able to bind all kinds of endotoxins from Gram-negative bacteria. The binding principle of EndoTrap® to LPS of Gram-negative bacteria is highly specific. EndoTrap® is not an antibody, not a synthetic peptide and not Polymyxin B based.

How can I use EndoTrap®?

EndoTrap® can be used either in column or batch mode. Generally column mode is easier to handle and more efficient in comparison to batch 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 is batch mode?

Chromatography is traditionally made in two modes: batch (or discontinuous) and continuous (column mode) chromatography. 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 best removal rate of endotoxin.

What is column mode?

Columns are easily prepared by packing with the depyrogenated EndoTrap® resin as 50% slurry in sterile buffer.

How to work with a real flow through system?

EndoTrap® is a **real flow through** system ("ready-to-use" columns). You can apply your sample (up to 50 ml with EndoTrap® blue/red) continuously to a 1 ml column – up to 3 ml at once. If desired you can additionally purchase a funnel, which enlarges the loading volume to 20-25 ml. We recommend regenerating the column after each cleaning step. One cleaning step means that your complete working volume passed through the column once. Afterwards you can fill in your sample volume again.

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

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

Is EndoTrap compatible with fully automated liquid chromatography systems?

Yes it is. You can fill the EndoTrap® resin in endotoxin free liquid chromatography columns. (The empty liquid chromatography columns are not available at Hyglos) If you want to use liquid chromatography systems with the original EndoTrap® columns you have to provide suitable adaptors. Unfortunately Hyglos cannot provide such equipment. Take care that the run time does not exceed 1 ml/min otherwise the gel bed will be compressed (EndoTrap® blue/red) With EndoTrap® HD higher flow rates are possible.

How to calculate the number of required cleaning steps (EndoTrap® blue/red)?

EndoTrap® can be reused at least three times (in general 10 times) without loss of endotoxin removal efficiency. In case your starting endotoxin concentration is very high or in case you wish to reach a very low concentration, EndoTrap® can be applied in a consecutive manner several times. Each round of application theoretically yields a two log reduction of endotoxin.

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, furthermore the performance may vary with the sample properties, especially of proteins.

Depending on your LPS starting concentration [EU] you must perform a certain number of cleaning steps, in order to achieve your desired LPS end concentration [EU]. To achieve best results and to be able to calculate which package size you need for your desired performance, **total LPS units applied should not exceed 30-50% of the maximum resin capacity.**

Starting LPS concentration (buffer) [EU]	1. cleaning step	regeneration step (kit includes regeneration buffer RB)	2. cleaning step	regeneration step (kit includes regeneration buffer RB)	3. cleaning step
100.000	1.000		10		0.1
10.000	100	1	0.01	0.01	0.01
1.000	10	0.1	<0.005	<0.005	<0.005
100	1	0.01	<0.005	<0.005	<0.005
10	0.1				
1	0.01				
0.1	<0.005				

LPS removal from **buffers**: With repetitive use of EndoTrap®, you can achieve concentrations as low as 0.005 EU/ml.

LPS removal from **proteins**: With repetitive use of EndoTrap®, decrease to concentrations as low as 0.1 EU/ml is possible. As it is a biological system, the efficiency of EndoTrap® slightly decreases at low endotoxin contamination levels. At 0.1 EU/ml the removal efficiency is approximately 70%.

If you do not yield your desired endotoxin level after the third cleaning step (each cleaning step ends with a regeneration step), please contact us and use our technical support!

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

The void volume of 1-ml-EndoTrap®-column is 0.3-0.5 ml, the sample will elute immediately after that volume has passed through the column. If there is a slight interaction between sample and column material and elution is delayed we recommend washing with equilibration buffer and collecting fractions. Pool fractions that contain your target material.

What can I do if the flow through rate is exceptionally slow?

Gravity flow guarantees a flow rate of about 0.5 ml/min. A solution of 10 ml usually needs 20 minutes to pass through a 1-ml-column. In case of a much lower flow rate, please check if there are bubbles in the column. During delivery and transport bubbles may emerge in the column. Removing bubbles from EndoTrap® columns is very easy: There are several possibilities. The first way is centrifuging the closed column at ~ 1200 rpm for five minutes. The second way is to press slightly against the frit on the top level with a pyrogen-free pipette. Another possibility to remove bubbles is to put a tube onto the end of the column and to connect it with a 5-ml injection. Make sure that the column is open and that there is buffer in the column. With the help of the injection you can aspirate bubbles.

Can EndoTrap® be used at 4 °C?

Yes, EndoTrap® is fully functional at 4 °C as well as at room temperature.

Is it possible to use EndoTrap® combined with chaotropic substances e.g. urea?

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

Are there inhibitory agents known for EndoTrap®?

EndoTrap® blue is inhibited by EDTA and other calcium-chelators as EGTA, HEDTA, NTA, Citrate, Ammoniumsulphate, SDS and other detergents. EndoTrap® red is inhibited by salt concentrations exceeding 250 mM, GdnHCl, Ammoniumsulphate, SDS and other detergents.

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

EndoTrap® blue and EndoTrap® HD can be used with DTT (up to 10 mM) or be pre-washed with DTT before use. EndoTrap® red is not tested yet.

Is it possible to use EndoTrap® at high ionic strength?

At this point it is important to distinguish between the two EndoTrap® systems: EndoTrap® blue and EndoTrap® HD can be used up to 600 mM NaCl without any significant loss of endotoxin removal efficiency. EndoTrap® red is **not** appropriate for high ionic strength it can be used only up to 250 mM NaCl.

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

EndoTrap® can not be autoclaved, but you can wash the resin with 30% Ethanol.

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

What is the principle of EndoTrap®?

EndoTrap® is an affinity matrix system. EndoTrap® can be used in either batch or chromatography mode.

How high is the binding capacity of EndoTrap®?

EndoTrap® blue /red: 2×10^6 endotoxin binding sites per ml (1 EU = 100 pg LPS)

EndoTrap® HD: 5×10^6 endotoxin binding sites per ml (1 EU = 100 pg LPS)

Which types of substances and in which concentration / volume can I apply to the EndoTrap® 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 molecular weight of proteins or substances. Substances such as proteins, peptides or antibodies are possible. EndoTrap® removes endotoxin from proteins with isoelectric points (pI) from 5 to 9.

EndoTrap® blue and HD work also with DNA (tested for plasmid DNA and also for RNA).

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

1 to 10 column volumes usually work best. Up to 50 ml can be applied onto a 1 ml EndoTrap® column without loss of endotoxin removal efficiency (1 column volumes "ready-to-use" = 1 ml).

For EndoTrap® HD we recommend a maximum LPS load of 2.5×10^6 EU/ml resin.

Which final endotoxin level can be achieved with EndoTrap®?

With repetitive use of EndoTrap®, you can go down to 0.005 EU/ml (sample: **buffer**).

With repetitive use of EndoTrap®, you can go down to 0.1 EU/ml (sample: **protein**).

With repetitive use of EndoTrap®, you can go down to 0.01 EU/ml (sample: **DNA**).

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, furthermore the performance may vary with the sample properties, especially of proteins.

Can EndoTrap® be regenerated?

Yes, with the provided regeneration buffer. The EndoTrap® system can be reused at least three times (in general 10 times) without loss of endotoxin removal efficiency.

Be aware to store EndoTrap® in regeneration buffer enriched with sodium azide to avoid growth of bacteria 0.02 %.

What are the main advantages of EndoTrap® in comparison to other products?

EndoTrap® has a highest protein recovery and endotoxin removal rate, it is an easy to use flow-through system. It also has a very stable performance at a wide variety of conditions (pH and ionic strength [salt]). Finally it is non toxic (not Polymyxin B based), for regulatory purposes, toxicity data, a Regulatory Support File and a Leakage ELISA (EndoTrap® HD/blue) are available.

Does the EndoTrap® 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® binds to a conserved region of the inner core of the lipopolysaccharide (LPS) molecule and can thereby bind to all kind of endotoxins from Gram-negative bacteria.

Does the EndoTrap® system bind yeast pyrogens?

No, because the yeast cell wall is completely different from the outer membrane of Gram-negative bacteria. Nevertheless in numerous cases it makes sense to clean yeast extracts with EndoTrap® because bacterial species occurring in laboratory water supplies are common sources for endotoxin contamination during downstream processes. LPS from most of these water contaminants is efficiently removed by EndoTrap®.

Hints & Tricks

My Endotoxin level is very high after using EndoTrap®. What has happened?

- LAL-assay is prone to errors.
- EndoTrap® blue/HD need some Ca^{2+} (about 50 – 100 μM) in solution. Are there any calcium binders in the used buffer such as EDTA, citrate, phosphate?
- Contaminated buffers – please check all buffers for ET contamination.
- Endotoxin contaminants on the EndoTrap® column: please do not forget to wash the EndoTrap® column with buffer before applying your sample. Test wash fraction for endotoxin.
- (Endotoxin levels of supplied materials have been tested and are under the detection limit. Equilibration, regeneration buffers and the flow path of the EndoTrap® column are tested for the presence of endotoxins. All endotoxin concentrations in our EndoTrap® products lie below 0.02 EU/ml)
- PBS buffer: Ca^{2+} ions are necessary for efficient binding to EndoTrap® blue/HD. Phosphate and Ca^{2+} form insoluble complexes and will precipitate. So EndoTrap® red may be the better choice in your case. When you are using PBS buffer with EndoTrap® blue/HD due to particular needs we recommend the following procedure:

Add Ca^{2+} freshly to your buffer. This means you add Ca^{2+} (we recommend CaCl_2) and start **immediately** with the endotoxin removal procedure. For efficient LPS binding on EndoTrap® blue/HD columns your buffer has to contain 50 to 100 μM Ca^{2+} .

Optionally you may also use **magnesium instead of calcium**. The LPS binding efficiency is almost the same but the precipitation is weaker.

What do I have to consider while working with proteases?

Proteases may destroy the EndoTrap® ligand during LPS removal. Please perform the cleaning steps at conditions where your protease is less active, e.g. 4°C, or change the buffer composition if possible.

Example: If you work with pepsin, we recommend to work above pH 6 since pepsin is an acidic protease.

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. Please contact us directly (see contact details below) and please provide the technical information requested for on page 9.

How can I increase the efficiency of endotoxin removal?

To reach better 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).

What can I do if I am not satisfied with the performance of EndoTrap®?

Please consider the chemical characteristics of your sample before choosing one improvement step.

Problem	EndoTrap® blue / EndoTrap® HD	EndoTrap® red
... bad sample recovery rate ...		
- due to ionic interactions	Increase the NaCl concentration of the equilibration / customer specific buffer up to 500 mM.	EndoTrap® red is not suitable for high ionic strength – please try EndoTrap blue.
- due to interactions with lipopolysaccharides	Hydrophobic interaction of your sample with LPS might be possible. As lipopolysaccharides form aggregates, it might be also possible that your sample arranged within these aggregates. It may help to disintegrate the aggregates or to reduce their size. For that purpose you can use Triethylamine (combined with 15 min ultrasonic treatment) or detergents. EndoTrap is suitable for buffers containing 20% glycerol. Note: Detergents may interfere with endotoxin detection in the LAL assay. The final concentration of Triethylamine and Tween20® should not exceed 0.5% and 0.005%, respectively.	
- due to your samples' negative charge	EndoTrap® blue/ HD ligand should not interact with negative charge.	EndoTrap® red ligand could interact with negative charge – please try EndoTrap® blue.
- due to interactions with calcium	If you work with calcium binding proteins, enrich your buffer with 1mM CaCl ₂ (<i>because EndoTrap® blue needs free calcium ions for the LPS-binding</i>).	EndoTrap® red does not need calcium ions – therefore you should not have this kind of problems.
... bad LPS removal rate ...		
- due to depletion of calcium	If no Calcium is present in the customer specific buffer, add 50-100 µM Ca ²⁺ (e.g. CaCl ₂)	EndoTrap® red does not need calcium ions – therefore you should not have this kind of problems.
- due to interference with buffer additives	Chelators of divalent cations (like EDTA, EGTA, Acetate- or Citrate buffers) have to be avoided.	Add up to 40% Ethanol to the equilibration buffer or customer specific buffer.
		Decrease the concentration of NaCl (or similar salts) in the customer specific buffer to 20 mM.
- due to limiting contact time	<ol style="list-style-type: none"> 1. Column mode: Use half of your sample or use a bigger column. 2. Batch mode: Increase the contact time or the EndoTrap® to sample ratio. 	
- due to limiting LPS binding capacity	To achieve best results, total LPS units applied should not exceed 30-50% of the maximum column capacity.	
... slow flow through rate (<< 0.2 – 1 ml/min [gravity flow]) ...		
- due to viscous solutions	EndoTrap is not suitable for viscous solutions!	
- due to bubbles	Remove bubbles by centrifuging the closed column (filled with buffer by a height of 1-2 cm) at ~ 1000 x g for 5 min. (using a "clinical-type" centrifuge, i.e. one with swinging baskets works best). For this procedure please place the column into a suitable 50 ml tube.	

If you have any problems please do not hesitate to contact our technical service team at inquiry@hyglos.de. Thank you!

Technical Support / Product Recommendation / Protocol Adaptation

If you need technical support relating to the use of EndoTrap® please answer the following questions and send the completed form back to us. If you are not able/ allowed to answer a question please skip to the next one.

1. EndoTrap® product (blue or red?):
2. Lot number of kit/column:
3. Short description of the problem (bad recovery rate, bad LPS removal, ...):

4. LPS contamination / concentration (EU/ml or EU/mg):
 - before using EndoTrap:
 - after using EndoTrap:
5. Bacteria strain:
6. Substance that should be cleaned (e.g. name of protein, antibody, ...):

7. Physical and/or chemical (e.g. LPS-binding protein) characteristics (of the sample):

8. Concentration (sample) before removal of endotoxins:
9. Recovery rate (or concentration of the sample) after removal of endotoxins:
10. Volume (sample):
11. Buffer composition (if you used your own buffer):

12. pH buffer (if you used your own buffer):
13. LAL assay (e.g. name, manufacturer):

Thank you for your cooperation!

Our Technical Support Team is always at your service and will contact you as soon as possible.

EndoTrap® HD:

For **biomanufacturing process** we recommend EndoTrap HD. EndoTrap HD has been especially optimized for application in biomanufacturing processes. It can be used in **early or late** biomanufacturing process steps. EndoTrap HD is based on a **hydrophilic, dimensionally stable affinity matrix** with excellent pressure / flow characteristics. Also a Regulatory Support File can be provided. EndoTrap HD is only available as slurry.

Hyglos Service: endotoxin removal and endotoxin detection

Hyglos offers an endotoxin removal and also an endotoxin detection service.

Please inquire for our services.

For inquiries and technical support please contact:

Hyglos GmbH, Am Neuland 3, 82347 Bernried, Germany

tel +49(0)8158 9060 0, fax +49(0)8158 9060 210, inquiry@hyglos.de

If you like to learn more about our products and services, please visit our website www.hyglos.de

Trademarks:

EndoTrap® is a registered international trademark of Hyglos GmbH

Pall Supor® is a trademark of Pall Corporation

Tween20® is a registered trademark of ICI America, Inc.

EndoTrap® patented technology has been developed and manufactured by Hyglos GmbH and is provided for research and bio-manufacturing use only.

Copyright: All contents, graphics, forms and programmes are subject to copyright 2009 of Hyglos, unless stated otherwise. The reproduction, alteration, use or dissemination of the information published here without the written permission of Hyglos GmbH is prohibited.