

Short Introduction into the EndoTrap®-family

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® 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.

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

	EndoTrap® blue / EndoTrap® HD	EndoTrap® red
▪ pH (buffer)	pH 4-9	pH 6-9
▪ Ionic strength	up to 600 mM NaCl	up to 250 mM NaCl <i>we recommend < 100 mM NaCl</i>
▪ suitable with EDTA, and other Calcium chelators containing buffers	No	Yes
▪ Customer specific equilibration buffer have to be enriched with calcium / magnesium	Yes	No
▪ PBS can be used as equilibration buffer	Yes, when enriched freshly with 50-100 µM Ca/Mg ²⁺ !*	Yes
▪ Suitable for subsequent endotoxin detection with EndoLISA®	No	Yes

* Further information about the preparation of buffers for EndoTrap® blue (EndoTrap® HD) you find in the FAQs EndoTrap®blue "proteins, medium and buffers" (FAQs are available on our homepage www.hyglos.de)

The following characteristics underline the outstanding performance of EndoTrap®

Rapidity	No incubation step – real flow-through system
Best protein recovery rate	Minimized sample loss, up to 99 % sample recovery
LPS variability	Endotoxin removal from broad range of bacteria strains
Reusability / regeneration	Re-usable at least three times, EndoTrap® HD at least 10 times without loss of endotoxin removal efficiency; - regeneration buffer included, the regeneration substance is non toxic, no Deoxycholate (DOC)*!
Temperature stability	Regular use in range between 4°C and room temperature (up to 35°C)
Buffers	Buffers included in kit (EndoTrap blue/red) buffer preparation protocol provided (EndoTrap HD)
Kit included (no extra cost for buffers!)	EndoTrap blue/red: ready to use columns or resin, regeneration and equilibration buffers EndoTrap HD: resin without buffers, preparing instructions for individual use provided, buffers can be supplied on request

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 if stored correctly at least 2 years from production date.

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

	EndoTrap® blue / EndoTrap® HD	EndoTrap® red
Regeneration buffer (included, except for EndoTrap® HD)	Regeneration buffer "blue" (based on "HEPES-buffer", pH 7.5) (endotoxin concentration < 0.02 EU/ml) Regeneration buffer "HD" (based on "TRIS-buffer", pH 8.0)	Regeneration buffer "red" (based on phosphate-buffer , pH 7.4) (endotoxin concentration < 0.02 EU/ml)
Equilibration buffer (included, except for EndoTrap® HD)	Equilibration buffer "blue" ("HEPES-buffer", pH 7.5) (enriched with 0.1 mM CaCl₂) (endotoxin concentration < 0.02 EU/ml) Equilibration buffer "HD" Customer specific, e.g. TRIS, HEPES, PBS, MOPS (enriched with 0.1 mM CaCl₂)	Equilibration buffer "red" phosphate-buffer , pH 7.4 (with only 80 mM NaCl) (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 and EndoTrap HD successful with HEPES, BORATE, TRIS, MOPS, MES, PIPES (PBS possible) (binding of EndoTrap® blue/HD to LPS depends on Ca/Mg ²⁺ , buffers have to contain at least 50-100 µM free Ca/Mg ²⁺)	We tested EndoTrap® red successful with PBS, HEPES, BORATE, TRIS, MOPS, MES, PIPES, Citrate-, Acetate-, Glycine- and Carbonate-buffers

- * (Sodium) Deoxycholate would have cytotoxic effects on cell culture and also influences cell growth and morphology of the cells. It is also reported that deoxycholate can induce DNA damages.

	EndoTrap® blue / EndoTrap® HD	EndoTrap® red
Tested type of substance which can be applied onto the column	<ul style="list-style-type: none"> ▪ proteins ▪ peptides ▪ antibodies ▪ plasmid DNA / RNA 	<ul style="list-style-type: none"> ▪ proteins ▪ peptides ▪ antibodies
pl of applied proteins	pl from 5-9	pl from 5-9
pH (buffer)	pH 4-9	pH 6-9
Ionic strength	up to 600 mM NaCl	up to 250 mM NaCl we recommend < 100 mM NaCl
Recommended working concentration of applied substances	EndoTrap blue: 1-10 mg/ml EndoTrap HD: 1 -20 mg/ml	1-10 mg/ml
Recommended sample volume	EndoTrap blue: up to 50 ml/ml resin EndoTrap HD: max. LPS load 2.5 mio EU/ml resin	up to 50 ml/ml resin
<u>Tested</u> substances which do not interfere with the performance of EndoTrap®*	up to 10 mM DTT (Dithiothreitol) 0.005 % Tween20 max. 0.005 % NaDOC max. 0.5 M GdnHCl 10% DMSO 20% Isopropanol 20% Methanol 20% Ethanol 10% Glycerol / Glycerine 0.5 urea (up to 2 M possible at ph 7) 300 mM Imidazol	DTT not tested max. 0.005 % NaDOC 20% DMSO 20 % Isopropanol 40% Methanol 20% Ethanol 20% Glycerol / Glycerine 1 M Urea 300 mM Imidazol
<u>Tested</u> substances which interfere with the performance of EndoTrap®*	<ul style="list-style-type: none"> ▪ > 10 mM NaOH ▪ SDS and other detergents ▪ Citrate ▪ ETDA, and other Calcium chelators (EGTA, HEDTA, NTA) 	<ul style="list-style-type: none"> ▪ SDS , Tween20 and other detergents ▪ GdnHCl ▪ Ammoniumsulphate
<u>Tested</u> kind of LPS (bacteria strain)	<ul style="list-style-type: none"> ▪ Ammoniumsulphate ▪ 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 	<ul style="list-style-type: none"> ▪ Escherichia coli K12 ▪ Salmonella enterica ▪ Citrobacter freundii ▪ Pseudomonas aeruginosa
<i>[for detailed information please read our FAQs or inquire for the package insert]</i>		for <ul style="list-style-type: none"> ▪ Klebsiella pneumoniae ▪ Serratia marcescens we recommend EndoTrap red!

* Please consider indicated concentration and substances relate to the performance of EndoTrap®. Some of these substances which can be used during the cleaning with EndoTrap® are not suitable for the LAL test. Please see instructions LAL test for further information.

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

Customer feedback

Statements from our customers. Please see also our publication list at www.hyglos.de

- ... "probably the best system of recent years".
- "It is a very good product"
- "easy to use, can be used in any lab without additional equipment"
- "very good, clearly a product that was needed in the market"
- "good helper for removing endotoxin"
- "fast and efficient method"
- "very effective"
- "good product, would recommend it to other colleagues"
- "it works, it's convenient, the service is excellent"

Hyglos Endotoxin detection Assay

Hyglos offers an endotoxin detection assay. If you like to learn more about our endotoxin detection assay EndoLISA®, please visit our website at www.endolisa.com.

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