



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.

The EndoTrap family contains two members:

EndoTrap HD

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 1 mM Ca/Mg²⁺ and 1 mM Citrate. Furthermore, it was particularly developed for challenging samples and large scale endotoxin removal in *e.g* biopharmaceutical production processes. For EndoTrap HD, a Regulatory Support File (RSF) and an EndoTrap leakage ELISA are available additionally.

EndoTrap red

especially for the use with buffers containing calcium chelators.

This multipurpose product portfolio covers a broad range of conditions with no special equipment or buffer requirements. EndoTrap HD 1/1 and 5/1 as well as all EndoTrap 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 HD and EndoTrap red, please, read our FAQs or inquire for 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 HD	EndoTrap red
▪ pH (buffer)	pH 4-10	pH 6-9
▪ Ionic strength	up to 1000 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 1 mM Ca ²⁺ /Mg ²⁺ and 1 mM Citrate pH 7.	Yes, but best results can be achieved with "half-concentrated" PBS



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Customer specific buffers can be used for equilibration and endotoxin removal. The composition should be adapted to the EndoTrap® system (HD or red), if necessary (see below).

EndoTrap® HD	EndoTrap® red
<p>Endotoxin removal with EndoTrap® blue works effectively in the pH range of 4-9 and NaCl concentrations of 50-600 mM.</p> <p>Buffers such as HEPES, TRIS, MOPS, MES, and PIPES are recommended. Citrate buffers and chelators of divalent cations (like EDTA) have to be avoided.</p> <p>Note: Customer specific buffers must contain 0.1 - 1 mM Ca²⁺ or Mg²⁺. When using PBS buffer always add 1 mM Ca²⁺ (e.g. CaCl₂) and 1 mM Citrate pH 7 freshly to the customer specific buffer especially. Otherwise phosphate and Ca²⁺ form an insoluble complex and will precipitate.</p> <p>For DNA application the following buffer composition is recommended: 10 mM Tris-HCl, 1 mM CaCl₂, 300 mM NaCl, pH 8.0</p>	<p>Endotoxin removal with EndoTrap® red works effectively in the pH range of 6-9 and NaCl concentrations in the range of 50-250 mM.</p> <p>Buffers such as HEPES, PBS, TRIS, MOPS, MES, PIPES and also Citrate, Acetate, Glycine and Carbonate buffers are recommended. EndoTrap® red can be used with chelators of divalent cations (like EDTA).</p> <p>Phosphate buffers with NaCl concentrations below 100 mM are recommended for EndoTrap® red.</p> <p>With a “classical” PBS buffer (10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 137 mM NaCl, 2.7 mM KCl, pH 7.4), the LPS removal rate will be ~97% for each cleaning step. With “half-concentrated”, PBS buffer LPS removal rates of ~99% can be achieved.</p> <p>Therefore dilute the PBS buffer 1:2 with endotoxin free water (5 mM Na₂HPO₄, 0.9 mM KH₂PO₄, 68.5 mM NaCl, 1.35 mM KCl, pH 7.4).</p>

Comparison of the specifications of EndoTrap blue/EndoTrap HD & EndoTrap red

	EndoTrap HD	EndoTrap red
<u>Tested substances which can be applied onto the column</u>	<ul style="list-style-type: none"> ▪ proteins ▪ peptides ▪ antibodies ▪ antigens ▪ plant extracts ▪ plasmid DNA / RNA 	<ul style="list-style-type: none"> ▪ proteins ▪ peptides ▪ antibodies
<u>Regeneration buffer (endotoxin concentration)</u> ← 0.02 EU/ml	Regeneration buffer “blue” (based on “HEPES buffer”, pH 7.5)	Regeneration buffer “red” (based on “Phosphate buffer”, pH7.4)
<u>Equilibration buffer (endotoxin concentration)</u> ← 0.02 EU/ml	Equilibration buffer “blue” (“HEPES buffer”, pH 7.5 enriched with 0.1 mM CaCl ₂)	Equilibration buffer “red” (“Phosphate buffer”, pH 7.4 with 80 mM NaCl)
<u>Other suitable equilibration buffers</u>	HEPES, Borate, TRIS, MOPS, MES, (PIPES and PBS possible when 1 mM Ca ²⁺ or Mg ²⁺ and 1 mM Citrate pH 7 is freshly added.)	PBS, HEPES, Borate, TRIS, MOPS, MES, PIPES, Citrate, Acetate, Glycine and Carbonate buffers
<u>pI of applied proteins</u>	pI from 5 - 9	pI from 5 - 9
<u>pH (buffer)</u>	EndoTrap HD: pH 4-10	pH 6 - 9



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	EndoTrap HD	EndoTrap red
<u>Ionic strength</u>	up to 600 mM NaCl	up to 250 mM NaCl < 100 mM NaCl is recommended
<u>Recommended working concentration of applied substances</u>	1 - 20 mg/ml	1 - 10 mg/ml
<u>Recommended sample volume per ml resin</u>	up to 50 ml/ml resin or 2.5*10 ⁶ EU LPS load/ml resin	up to 50 ml
<u>Tested substances which do not interfere with the performance of EndoTrap®*</u>	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 / Glycerin 0.5 M Urea (up to 2 M at pH 7 possible) 300 mM Imidazole	DTT not tested max. 0.005% NaDOC 20% DMSO 20% Isopropanol 40% Methanol 20% Ethanol 20% Glycerol / Glycerin 1 M Urea 300 mM Imidazole
<u>Tested substances which interfere with the performance of EndoTrap® and therefore having an inhibitory effect on LPS binding</u>	<ul style="list-style-type: none"> ▪ > 10 mM NaOH ▪ SDS and other detergents ▪ Citrate ▪ EDTA, and other Calcium chelators (EGTA, HEDTA, NTA) ▪ Ammoniumsulphate 	<ul style="list-style-type: none"> ▪ > 250 mM NaCl ▪ SDS, Tween20 and other detergents ▪ GdnHCl ▪ Ammoniumsulphate
<u>Tested LPS-types (bacterial strain)</u>	<ul style="list-style-type: none"> ▪ <i>Escherichia coli</i> K12, BL21, ▪ R1, R2, R3, R4, ▪ <i>Salmonella enterica</i> ▪ <i>Citrobacter freundii</i> ▪ <i>Citrobacter amalonaticus</i> ▪ <i>Citrobacter koseri</i> ▪ <i>Pseudomonas aeruginosa</i> ▪ <i>Pseudomonas stutzeri</i> ▪ <i>Enterobacter aerogenes</i> ▪ <i>Enterobacter asburiae</i> ▪ <i>Enterobacter cloacae</i> ▪ <i>Aeromonas hydrophilia</i> 	<ul style="list-style-type: none"> ▪ <i>Escherichia coli</i> K12 ▪ <i>Salmonella enterica</i> ▪ <i>Citrobacter freundii</i> ▪ <i>Pseudomonas aeruginosa</i> <p><i>Use EndoTrap red for:</i></p> <ul style="list-style-type: none"> ▪ <i>Klebsiella pneumoniae</i> ▪ <i>Serratia marcescens</i>

* 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. EndoLISA® endotoxin detection assay can be used as an alternative method.



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The following characteristics underline the outstanding performance of EndoTrap

Rapidness	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 HD 1/1, 5/1, EndoTrap red) buffer preparation protocol provided (EndoTrap HD)
Kit included (no extra cost for buffers!)	EndoTrap HD 1/1, 5/1, EndoTrap red : <i>ready to use</i> 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 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 red : Highly cross-linked 4% sepharose, spherical beads EndoTrap HD : hydrophilic, cross-linked methacrylic polymer
Void volume	0.3 to 0.5 ml / ml resin
Mean particle size	40 - 90 µm
Max. flow rate	EndoTrap red : 0.2 - 1 ml/min (gravity flow) EndoTrap HD : up to 100 ml/h/ml resin
Max. pressure	EndoTrap 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.

* (Sodium) Deoxycholate may show cytotoxic effects on cell culture and also influences cell growth and morphology of the cells. It is also reported that deoxycholate can induce DNA damage.



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Hyglos Endotoxin Detection Assays

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

Products of the EndoTrap - family

Product	Contents	Cat. No.
EndoTrap HD 1/1	1 x 1-ml column, ready to use, equilibration buffer, regeneration buffer, storage buffer	800053
EndoTrap HD 5/1	5 x 1-ml columns, ready to use, 5x equilibration buffer, 5x regeneration buffer, 5x storage buffer	800063
EndoTrap HD 10	10 ml settled resin, supplied as 50% slurry in regeneration buffer	800034
EndoTrap HD 50	50 ml settled resin, supplied as 50% slurry in regeneration buffer	800035
EndoTrap HD 250	250 ml settled resin, supplied as 50% slurry in regeneration buffer	800036
EndoTrap HD Bulk	Bulk resin, supplied as 50% slurry in regeneration buffer	800037
EndoTrap Leakage ELISA for EndoTrap HD	12 x 8 well stripes 1 vial POD-antibody 1 vial Standard, 20 ml ABTS substrate, ready to use	800033
EndoTrap red 1/1	1 x 1-ml column, ready to use, equilibration buffer, regeneration buffer	321053
EndoTrap red 5/1	5 x 1-ml columns, ready to use, equilibration buffer, regeneration buffer	321063
EndoTrap red 10	10 ml settled resin, supplied as 50% slurry in regeneration buffer; equilibration buffer, regeneration buffer	321064
EndoTrap red 50	50 ml settled resin, supplied as 50% slurry in regeneration buffer; equilibration buffer, regeneration buffer	321075



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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”

For inquiries and technical support please contact

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

Trademarks

EndoTrap® is a registered international trademark of bioMérieux SA

EndoLISA® is a registered international trademark of bioMérieux SA

EndoZyme® is a registered international trademark of bioMérieux SA

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 use only.

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