LIONEX Diagnostics and Therapeutics

Package Insert EndoTrap® HD FPLC column 5mL

Cat. No. LET-HD-FPLC5ML - EndoTrap® HD FPLC column 5mL

For laboratory and research use only. Not for use in diagnostic procedures.

Store at +2 to 8 °C

Table of Contents

1. General Information

1.1 Intended Use

EndoTrap® HD is intended for *in vitro* quantitative removal of lipopolysaccharide (LPS) from biological samples in aquatic solutions such as proteins, antibodies, cell extracts and nucleic acids. EndoTrap® HD can be used in small scale processes for R&D and large scale processes, like manufacturing. It can be applied in early or late biomanufacturing process steps.

EndoTrap® HD is based on hydrophilic, dimensionally stable affinity matrix with excellent pressure/flow characteristics. An EndoTrap® HD Leakage ELISA is available for the quantitative determination of the EndoTrap® ligand leakage. A Regulatory Support File (RSF) is provided on request.

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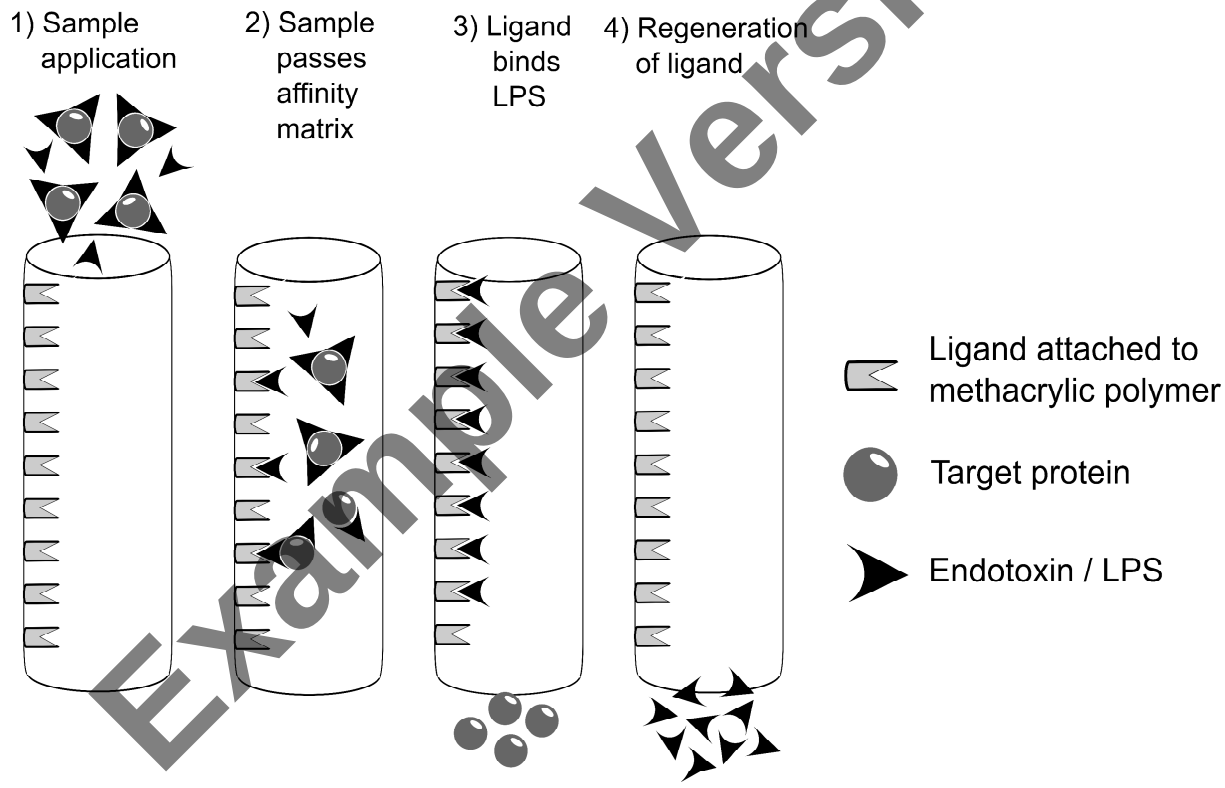
1.2 Principle



1.3 EndoTrap® HD FPLC column 5mL

Components
1 x EndoTrap® HD FPLC column 5mL

1.4 Specifications

Matrix		
LPS Binding Ligand	EndoTrap® HD ligand:	bacteriophage protein
	Protein structure:	homo-trimer
	Molecular weight:	150 kDa (trimer)
	Dissociation constant:	$K_D = 5 \times 10^{-8}$ M
	Isoelectric point:	8.52
Bead Matrix	Matrix:	hydrophilic, cross-linked methacrylic polymer
	Particle size range:	40 – 90 µm
	Exclusion limit:	5000 kDa (globular proteins) 1000 kDa (PEG)
	Mean pore diameter:	1000 Å
EndoTrap® HD	Binding capacity:	> 5×10^6 EU/mL resin (1 EU = 100 pg LPS)
	Operating pH range:	pH 4 - 10
	Operating flow rate:	automatic systems: maximum 600 to 840 cm/h
	Operating pressure:	up to 0.3 MPa is recommended (maximum pressure drop on column is 0.7 MPa)
	Temperature stability:	2 - 35 °C
	Ligand leakage:	< 20 ng/mL (from 10 mg/mL BSA)
	Shiptment condition:	ambient temperature
	Shelf life:	EndoTrap® HD (unused material) is stable until the stated expiry date when stored correctly (at 2 - 8 °C).
Cartridge		
	Format:	5 mL
	Inner diameter:	11 mm (0.039 in)
	Column body material:	Acrylate
	End plug material:	Polypropylene
	Inlet/Outlet:	10-32 UNF female thread
	pH stability:	2-14
	Max. Flow rate:	6 mL/min
	Recommended Flow rate*:	0.5 – 2.0 mL/min
	Recommended Operational pressure:	Up to 3 bar (43 psi)

* Dynamic binding capacity strongly correlates with the flow rate and other parameters such as protein size and buffer conditions

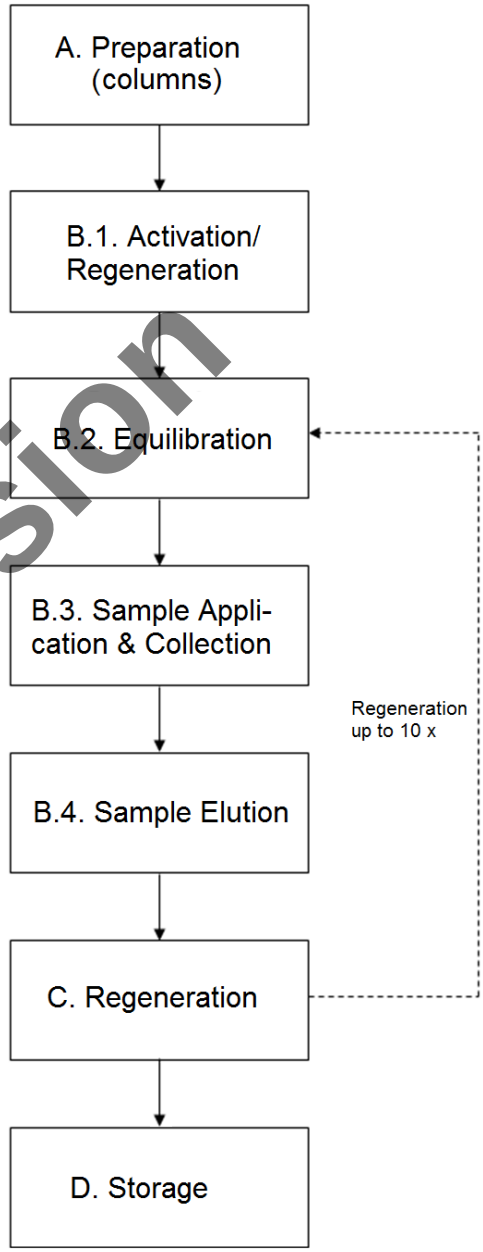
1.5 Precautions

- Custom specific equilibration and sample buffers used for endotoxin removal with EndoTrap® HD must contain **minimum 0.1 mM free Ca^{2+}**.
- EndoTrap® HD resin and columns are supplied with ProClin™ as preservative. For further information see the EndoTrap® HD Material Safety Data Sheet.
- All materials used, such as containers or pipette tips and buffers, must be endotoxin-free. Glass ware is preferred, as endotoxins can be destroyed by heat treatment (200 °C, 4 h or 250 °C, 1 h).
- Buffers should be prepared from endotoxin-free materials with endotoxin-free water.
- Buffers, resin and sample should have the same temperature (4-35 °C) during the processing steps.
- For proteases see page 11.
- EndoTrap® HD 5x buffers (Cat. No. LET0015, LET0016 and LET0017), also contained in EndoTrap® HD 1/1 (Cat. No. LET0009) and EndoTrap® HD 5/1 (Cat. No. LET0010) must be diluted 1:5 with endotoxin-free water prior to use.
- Proteases may destroy the EndoTrap® ligand during LPS removal. Please perform the cleaning steps at conditions where the protease is less active, e.g. 4 °C, or change the buffer composition if possible.

2. Protocol

A. Preparation

1. Place the column in a suitable holder and connect the columns to your FPLC / HPLC system. Please follow the instructions in table **1.4 Specifications**.
2. Wash with at least 4 column volumes of equilibration buffer or customer specific buffer (see 1.4 Specifications for recommended flow). Use pH monitoring to check the pH of the eluent.



B. Activation and Equilibration

1. Wash column with 5–10 column volumes of regeneration buffer until baseline, eluent pH and conductivity are stable.
2. Pre-equilibrate the column with 3 CV equilibration buffer* plus 1 M NaCl.
3. Equilibrate with at least 5–10 column volumes of equilibration buffer* or customer specific buffer.

C. Endotoxin removal**

1. Apply your **sample** (either **in equilibration buffer** or in customer specific buffer) and start collecting the fractions (depending on the applied sample volume). The applied sample elutes directly after the column void volume. Please note that **the first column volume** of a sample has a higher ligand leakage than the rest of the purified sample. To ensure the lowest ligand concentration in your sample we recommend collecting the first column volume separately.
2. In order to elute your entire sample, apply extra 1 column volumes **equilibration buffer** or customer specific buffer, and collect the flow through completely.

D. Regeneration***

1. Wash the column with at least 5-10 column volumes **regeneration buffer**. **Continue with step B.3**

E. Storage

Equilibrate the column with at least 5-10 CV **storage buffer** supplement with **2.5 ppm ProClin™ or 0.02% sodium azide** Insert the stoppers at inlet and outlet and store at 2 to 8 °C (shelf life until the indicated expiry date). Alternatively 20% ethanol can be used as storage buffer; the storage time will then be reduced to 4 weeks.

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* The Equilibration buffer should be identical with the sample buffer used for the process and has to contain 0.1 mM Ca^{2+} (e.g. $CaCl_2$).
** EndoTrap® HD works under a broad range of conditions, there are nearly no limits regarding pH, ionic strength and additives.
*** EndoTrap® HD can be regenerated under mild conditions by complexing Ca^{2+} with EDTA at increased ionic strength. Regeneration buffer: 20 mM HEPES, 1 M NaCl, 2 mM EDTA, pH 7.5
Volume: 6 column volumes.

Optional Steps

Endotoxin / LPS detection:
- Control the LPS removal efficiency using an endotoxin detection assay. If the LPS contamination is still too high, perform a second LPS removal step.

Protein polishing / recovery:
- Combine the fractions and filtrate the solution over 0.2 µm membranes to ensure sterile conditions.
- Measure the protein concentration with appropriate methods or measure the absorption at 280 nm.

3. Supplementary Information

3.1 Cleaning in Place (CIP), Sanitisation, ligand leakage

To ensure **low ligand leakage** starting the protocol with a regeneration step followed by an equilibration step is recommended, therefore the concentration of leaked ligand in fractions should be in the range of 300 pg/mL to 20 ng/mL.

- **The first column volume** of sample has a higher ligand leakage than the rest of the purified sample. To ensure the lowest ligand concentration in collecting the first column volume separately is recommended.
- When applying **concentrated sample solutions** (*e.g.* > 5 mg/mL), the concentration of leaked ligand could be higher than 10 ng/mL in the very first fraction.
- Leakage of minor amounts of ligand is typical for all affinity materials. We recommend controlling the leakage of the LPS-binding ligand with LIONEX' EndoTrap® HD Leakage ELISA

Cleaning in Place (CIP)	CIP removes tightly bound, precipitated or denatured substances from the purification system.	
	CIP buffer:	20 mM Tris, pH 8.0 supplemented with 6 M Urea or 2 M GdnHCl
	Protocol:	- Clean the column with 6 column volumes CIP buffer. - Wash immediately with at least 5 column volumes of equilibration buffer. Use reversed flow direction.
	Max flow rate:	600 to 840 cm/h
Sanitisation	Sanitisation reduces microbial contamination of the resin to a minimum.	
	Sanitisation buffer:	0.1 M Acetic acid + 20% Ethanol
Protocol	Incubate the column with sanitisation buffer for 2 to 12 hours	
Storage	Unused resin can be stored in the container. Ensure that the container is densely closed. EndoTrap® HD is supplied in 20 mM sodium phosphate, 150 mM NaCl, 2 mM EDTA, pH 7.4, 2.5 ppm ProClin™.	
	Unused material:	at 2-8 °C
	Regenerated material: at 2-8 °C in storage buffer, supplemented with 2.5 ppm ProClin™ or 0.02% sodium azide.	
	Note: **Do not freeze!**	
Scaling-up	After optimizing at laboratory-scale, the process can be scaled up. For this purpose some parameters have to be changed while others remain constant.	
	- Select bed volume according to required LPS binding capacity.	
	- Select column dimension so that high flow rates can be used.	
	- Select linear flow rate during sample application to ensure that the contact time is not shorter than established in the small scale study.	
	- Keep the sample concentration constant	

3.2 Custom Specific Equilibration Buffer

Table 2: Custom specific equilibration buffer: Some of the possible additives may interfere with the LAL assay.

Equilibration buffer	The column should be equilibrated with the same buffer which is used for the sample; the pH and different additives can be adjusted to the concentrations indicated in this table.	
	pH:	4-10
	Ionic strength:	50-1000 mM NaCl
	Calcium conc.:	0.1-10 mM Ca^{2+}
	Ca^{2+} (*e.g.* $CaCl_2$) has to be added freshly to **your** customer specific buffer	
	Possible additives:	up to 10 mM DTT (Dithiothreitol) 0.005% Tween20® max. 0.005% NaDOC max. 0.5 M GdnHCl 10% DMSO 20% Ethanol 10% Glycerol 2 M Urea 300 mM Imidazole
	Interfering substances:	> 10 mM NaOH SDS Ammoniumsulphate Citrate, ETDA and other Calcium chelators (possible when compensated equally with Ca^{2+})

3.3 Sample Application

Table 3: Samples to be applied to EndoTrap® HD.

Applied samples	All kind of complex biological solutions and purified components can be processed on EndoTrap® HD.	
	Sample materials:	proteins, peptides, antibodies, antigens, plant extracts, plasmid DNA/RNA
	Sample concentration:	1-20 mg/mL
	Sample volume:	50 mL per mL resin or $2.5*10^6$ EU LPS load per mL resin

3.4 Tested LPS Sources

Table 4: Tested LPS sources: Efficiency of LPS removal has been tested for various gram-negative bacteria strains.

Evaluated spectrum of EndoTrap® HD towards various LPS sources	*Escherichia coli* K12, R1, R2, R3, R4	*Pseudomonas stutzeri*
	Salmonella enterica	*Enterobacter aerogenes*
	Citrobacter freundii	*Enterobacter asburiae*
	Citrobacter amalonaticus	*Enterobacter cloacae*
	Citrobacter koseri	*Aeromonas hydrophila*
	Pseudomonas aeruginosa	

3.5 Sanitisation Test (only matrix)

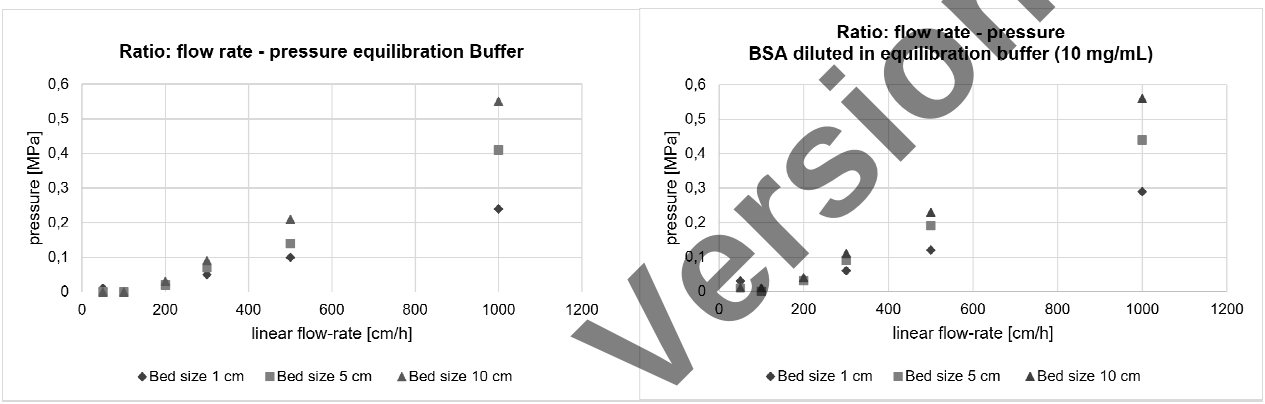
Table 5: Sanitisation test: Batch mode: Endotoxin removal of 1.5 mL endotoxin spiked BSA (20 mg/mL, 600 EU/mL) with 0.1 mL EndoTrap® HD resin. The indicated sanitisation buffer provides 100% reduction of bacterial contamination (10^7 CFU incubated for indicated time). Endotoxin removal is not affected when resin is exposed to the same buffers for 24 hours.

Sanitisation buffer	**Incubation time**	**Endotoxin removal efficiency [%]**	**Factor of reduction [CFU]**	
			Listeria	*E. coli*
0.1 M Acetic acid + 20% EtOH	4 hours	99.89	10^7	10^7
70% EtOH	6 hours	99.82	10^7	10^7
0.1 M HCl	6 hours	99.87	10^7	10^7

3.6 Pressure / Flow Comparison (only matrix)

Table 6: Pressure / flow comparison: The pressure / flow comparison between buffer (20 mM Hepes, pH 7.4; 150 mM NaCl, 0.1 mM $CaCl_2$) and BSA (10 mg/mL dissolved in buffer). The pressure / flow data were determined in Millipore Vantage column (diameter 16 mm, height 250 mm) packed to a bed height as indicated using equilibration buffer as the mobile phase at 20 °C.

	Bed size: 1 cm		Bed size: 5 cm		Bed size: 10 cm	
Flow rate [cm/h]	**Pressure [MPa]: buffer**	**Pressure [MPa]: BSA**	**Pressure [MPa]: buffer**	**Pressure [MPa]: BSA**	**Pressure [MPa]: buffer**	**Pressure [MPa]: BSA**
50	0.01	0.03	0	0.01	0	0.01
100	0	0	0	0	0	0.01
200	0.02	0.03	0.02	0.03	0.03	0.04
300	0.05	0.06	0.07	0.09	0.09	0.11
500	0.1	0.12	0.14	0.19	0.21	0.23
1000	0.24	0.29	0.41	0.44	0.55	0.56



3.7 EndoTrap® HD Buffer Composition

Table 7: EndoTrap® HD buffer composition: This table shows the composition of the non-concentrated EndoTrap® HD buffers. EndoTrap® HD 5x buffers have to be diluted 1:5 with endotoxin-free water prior to use.

Buffer	Composition
EndoTrap® HD Equilibration Buffer	20 mM HEPES, 150 mM NaCl, 0.1 mM $CaCl_2$, pH 7.5
EndoTrap® HD Regeneration Buffer	20 mM HEPES, 1 M NaCl, 2 mM EDTA, pH 7.5
EndoTrap® HD Storage Buffer*	20 mM Sodium Phosphate, 150 mM NaCl, 2 mM EDTA, pH 7.4

* EndoTrap® HD resin is delivered in storage buffer supplemented with 2.5 ppm ProClin(™). EndoTrap® HD Storage Buffer has to be supplemented with 2.5 ppm ProClin(™) or 0.02% Na-Azide prior to use.

3.8 Trouble Shooting Guide

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

Issue	Action
…low sample recovery rate…	
- due to **ionic interactions**	Increase the NaCl concentration of the equilibration / sample buffer. 150 to 250 mM NaCl should be sufficient.
- due to **interactions** with l**ipopolysaccharides**	Hydrophobic interaction of samples with LPS may occur. As lipopolysaccharides form aggregates, it might also be possible that your sample arranges within these aggregates. It may help to disintegrate the aggregates or to reduce their size. For that purpose Triethylamine (combined with 15 min ultrasonic treatment) or detergents can be used. Note: Detergents may interfere with endotoxin detection in the LAL assay.
…low LPS removal rate…	
- due to **depletion of calcium**	When working with calcium binding proteins, ensure that your equilibration / sample buffer contains at least 0.1 mM free Ca^{2+}. If using phosphate-based buffers add 1 mM Ca^{2+} and 1 mM Citrate ph7.
- due to interference with **buffer additives**	Chelators of divalent cations (like EDTA, EGTA, Acetat- or Citrate buffers) have to be avoided or compensated equally with free Ca^{2+}.
- due to limiting **contact time**	Increase contact time on the column. Time-on-the-column should be at least 30 seconds.
- due to limiting **LPS binding capacity**	To achieve best results, total LPS units applied should not exceed 30 to 50% of the maximum column capacity (5 x 10^6 EU/mL resin).
…low up-scaling results…	
- due to the **change of parameters**	Check, if parameters in “Operating EndoTrap® HD on Large Scale” (page 7 & 8) like endotoxin capacity, time-on-the-column and volume to be processed become limiting.
…slow flow through rate...	
- due to **viscous solutions**	For viscous solutions EndoTrap® HD is recommended in batch mode.

4. Technical Support and Further Product Information

4.1 Inquiries and Technical Support

Internet

Visit EndoTrap® on LIONEX website www.lionex.de.
For following details contact us.

Technical resources including manuals, application notes, Certificates of Analysis, Material Safety Data Sheets (MSDS), FAQs and references
Complete technical service contact information
Access to price lists and ordering forms
Additional product information and special offers

Contact us

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4.2 Legal Statements and Patent Information

Trademarks

EndoTrap® and EndoGrade® are licensed registered trademarks of LIONEX GmbH
ProClin™ is a registered trademark of Rohm and Haas Company
Tween20® is a registered trademark of ICI America, Inc.

Patent information

Parts of this product are protected under the following patents: EP1516188 and EP1695085

4.3 Related Products by LIONEX

EndoTrap® HD Leakage ELISA

- EndoTrap® HD Leakage ELISA for determination of EndoTrap® HD binding ligand

EndoGrade® Endotoxin-free Accessories

- EndoGrade® Glass Test Tubes - Endotoxin-free borosilicate glass test tubes with screw cap

EndoGrade® Endotoxin-free Reagents

- EndoGrade® Ovalbumin - Ultra-pure Ovalbumin for immunology and allergology research