LIONEX Diagnostics and Therapeutics

Package Insert EndoTrap® HD

- **Cat. No. LET0009 - EndoTrap® HD 1/1**
- **Cat. No. LET0010 - EndoTrap® HD 5/1**
- **Cat. No. LET0023 - EndoTrap® HD 5**
- **Cat. No. LET0011 - EndoTrap® HD 10**
- **Cat. No. LET0012 - EndoTrap® HD 50**
- **Cat. No. LET0013 - EndoTrap® HD 250**
- **Cat. No. LET0031 - EndoTrap® HD Buffer Kit**

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

Store the kits at +2 to 8 °C

Table of Contents

1. General Information

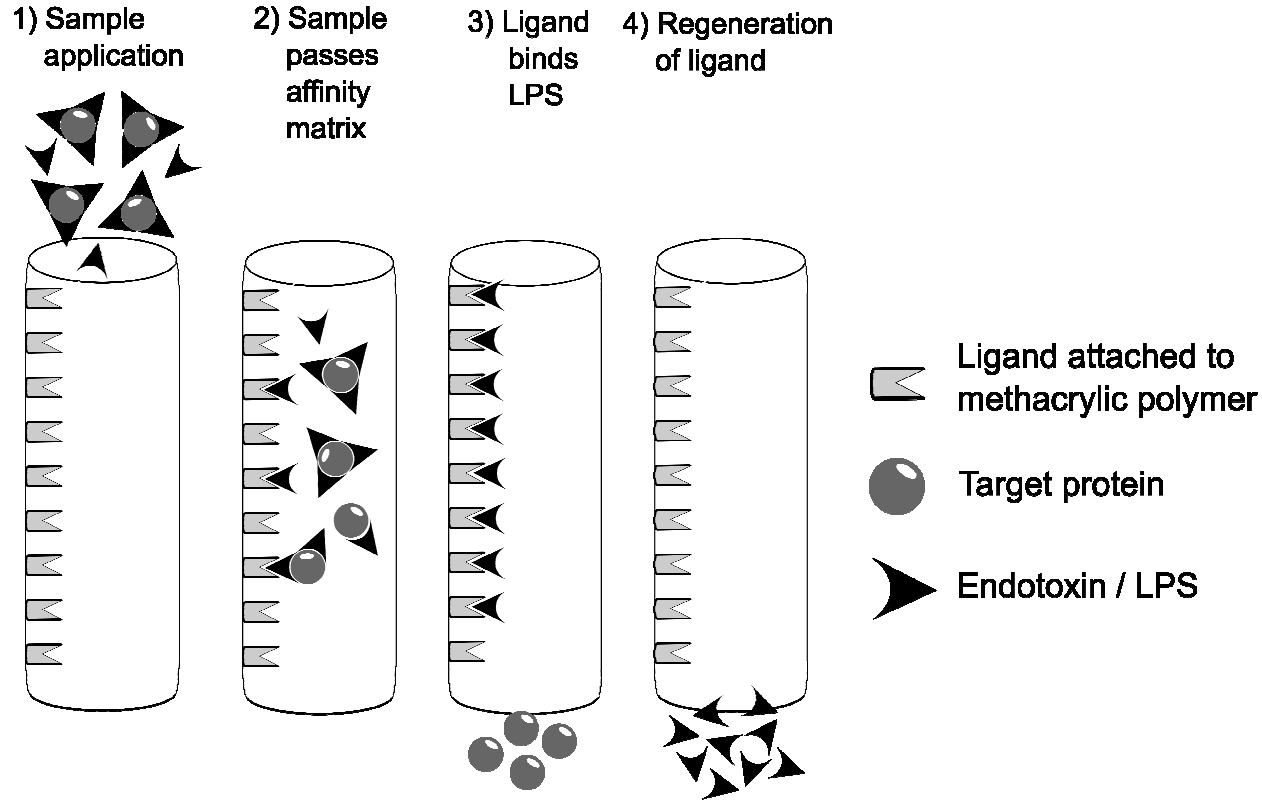
1.1 Intended Use

EndoTrap® HD is intended for *in vitro* quantitative removal of lipopolysaccharide (LPS) from biological samples in aqueous 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.

For laboratory and research use only.

1.2 Principle



1.3 EndoTrap® HD Kit Components

Kit Components	
EndoTrap® HD 1/1	**EndoTrap® HD 5/1**
1x1 mL EndoTrap® HD column	5x1 mL EndoTrap® HD column
25 mL EndoTrap® HD 5x Equilibration Buffer	125 mL EndoTrap® HD 5x Equilibration Buffer
25 mL EndoTrap® HD 5x Regeneration Buffer	125 mL EndoTrap® HD 5x Regeneration Buffer
25 mL EndoTrap® HD 5x Storage Buffer	125 mL EndoTrap® HD 5x Storage Buffer

EndoTrap® HD Buffer Kit:
125 mL EndoTrap® HD 5x Equilibration Buffer
125 mL EndoTrap® HD 5x Regeneration Buffer
125 mL EndoTrap® HD 5x Storage Buffer

Single components			
EndoTrap® HD 5	**EndoTrap® HD 10**	**EndoTrap® HD 50**	**EndoTrap® HD 250**
5 mL settled resin	10 mL settled resin	50 mL settled resin	250 mL settled resin

1.4 Specifications

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 small columns: 0.2 to 1 ml/min (gravity flow)
	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)
	Shipment condition:	ambient temperature
	Shelf life:	EndoTrap® HD (unused material) is stable until the stated expiry date when stored correctly (at 2 - 8°C). At room temperature the unused resin is stable up to 3 months. **Do not freeze!**

1.5 Important notes

- Custom specific equilibration and sample buffers used for endotoxin removal with EndoTrap® HD must contain **minimum 0.1 mM free Ca^{2+}**.

  Alternatively, minimum **0.1 mM Mg^{2+}** can be used.

- 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).
- Empty gravity flow columns and funnels are available from LIONEX and supplied **not endotoxin-free**. In order to exclude any co-contamination with LPS, empty columns and funnels should be treated with at least 1 M NaOH overnight (6 - 12 h), subsequently washed with endotoxin-free water and air dried. The protocol “Procedure for packing gel into a column” is available from LIONEX on request.
- 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.
- 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.
- 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.

1.6 Storage

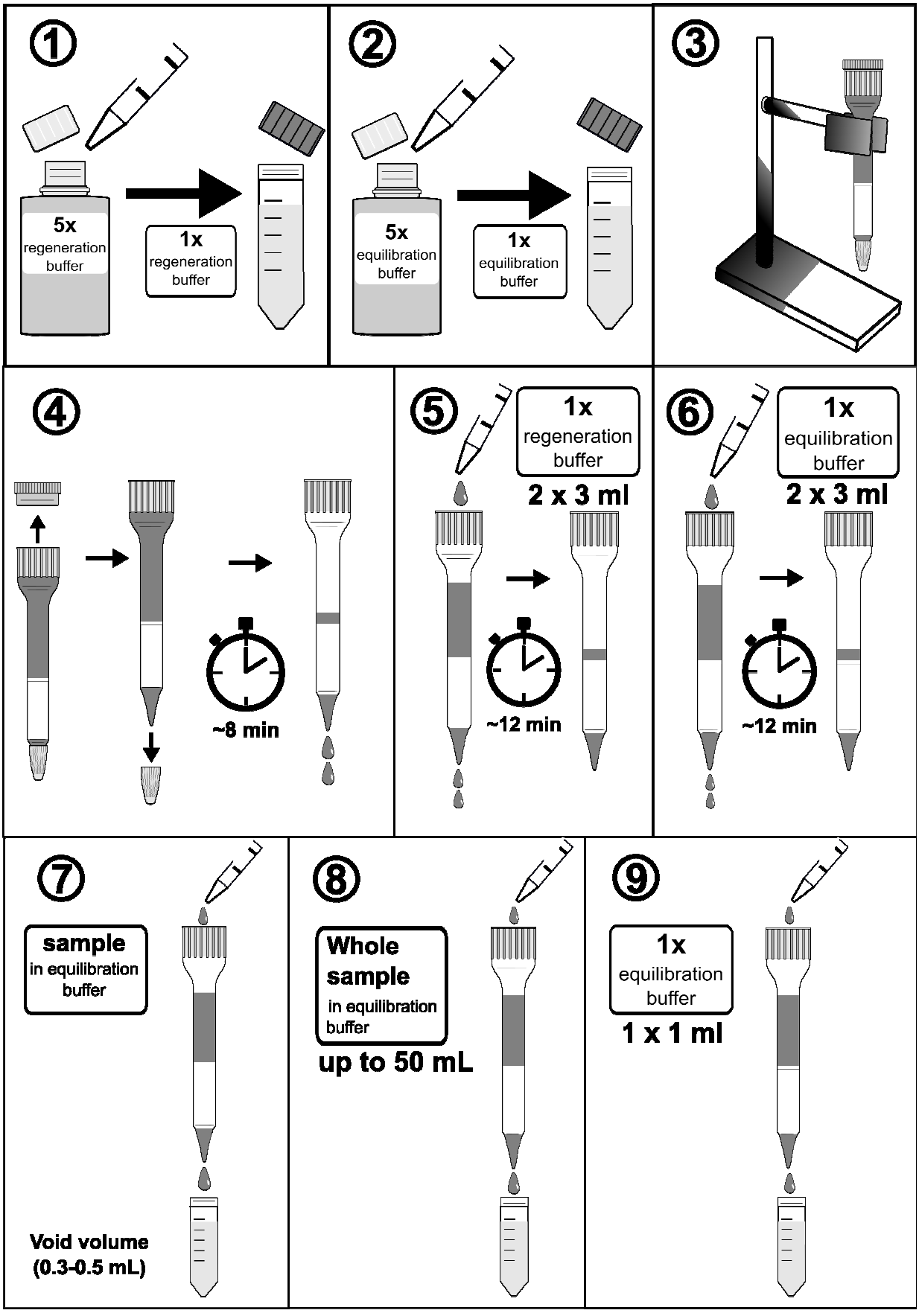
EndoTrap® HD resin and buffers should be stored at 2-8°C. After using the regenerated resin should be stored in storage buffer supplement with **2.5 ppm ProClin™ or 0.02% sodium azide** (shelf life until the indicated expiry date). Alternatively, 20% ethanol can be used as storage buffer; the storage time will then be reduced to 4 weeks.
At room temperature the fresh resin is stable up to 3 months.

1.7 Short protocol – Column mode

Notes before starting

- Custom specific equilibration and sample buffers used for endotoxin removal with EndoTrap® HD must contain **minimum 0.1 mM free Ca^{2+}**.
  Alternatively, **minimum 0.1 mM Mg^{2+}** can be used.
- A sample volume of 2 - 10 ml is recommended (the maximum sample volume is 50 mL).
- The sample concentration shall not exceed 1 mg per mL resin.
- To avoid the dilution of the sample smaller fractions can be collected and tested by protein assay.

1. Dilute 5x regeneration buffer with endotoxin-free water.
2. Dilute 5x equilibration buffer with endotoxin-free water [buffer must contain **Ca^{2+}/Mg^{2+}**].
3. Place column in a suitable holder.
4. First remove the top cap and then the bottom cap of the prepacked column. Allow the storage solution to drain from the column.
5. Fill the column with 3 mL **regeneration buffer** and let the column drain out completely. Repeat this step.
6. Fill the column with 3 mL **equilibration buffer** or customer specific buffer and let the column drain out completely. Repeat this step.
7. Apply your **sample** (either **in equilibration buffer** or in customer specific buffer) onto the column and let drain off the void volume of 0.3-0.5 mL.
8. Directly after the void volume the sample elutes and can be saved. A sample volume up to 50 mL can be applied.
9. To elute the entire sample the column can be rinsed with 1 mL equilibration buffer.


①
5x
regeneration
buffer
1x
regeneration
buffer
②
5x
equilibration
buffer
1x
equilibration
buffer
③
④
~8 min
⑤
1x
regeneration
buffer
2 x 3 ml
~12 min
⑥
1x
equilibration
buffer
2 x 3 ml
~12 min
⑦
sample
in equilibration
buffer
Void volume
(0.3-0.5 mL)
⑧
Whole
sample
in equilibration
buffer
up to 50 mL
⑨
1x
equilibration
buffer
1 x 1 ml

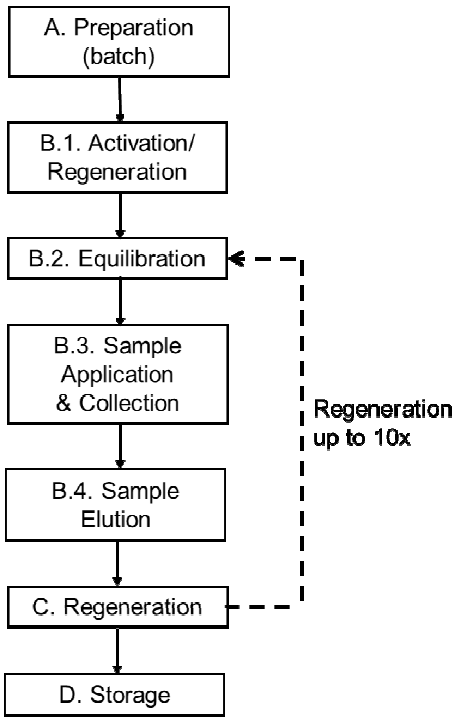
2. EndoTrap® HD Protocols

EndoTrap® HD can be used in column or batch mode. In general 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 and contact time may have to be optimized for each application to obtain maximum endotoxin removal with minimum product loss.
Should you want to pack the columns (small / large plastic columns) yourself, a protocol "Procedure for packing gel into a column" is provided at www.lionex.de (Downloads).
A protocol for HPLC / FPLC automated systems ("Application Protocol for Pilot Scale") is also provided when using own liquid chromatography systems at www.lionex.de (Downloads).

2.1 Protocol Batch Mode

A. Preparation

A ratio of 2:1 to 10:1 between sample and resin volume is recommended (up to 50 mL sample per mL resin is possible). All centrifugation steps should be carried out at **~ 3000 x g for 2 min** (bench top centrifuge)! Several contact times should be tested to determine the optimal contact time for endotoxin removal**.** Remove the storage buffer from gel slurry by centrifugation and discard the supernatant. 5x buffers have to be diluted 1:5 with endotoxin-free water prior to use.



B. Activation and Endotoxin Removal

1. Add 2 gel volumes of **regeneration buffer**[1], mix by gently shaking the tube for 5 sec; centrifuge, and discard the supernatant. Repeat twice.

2. Add 2 gel volumes of **equilibration buffer**[2] or customer specific buffer, mix by gently shaking the tube for 5 sec; centrifuge and discard the supernatant. Repeat twice.

3. Add the sample (either in **equilibration buffer** or in customer specific buffer) and incubate for at least 5 min. gently shake or rotate the tube while incubating.

4. Centrifuge at ~ 3000 x g for 2 min (bench top centrifuge) and transfer the supernatant (= sample) to an endotoxin-free tube.

C. Regeneration

Resuspend the EndoTrap® HD gel pellet in 2 gel volumes of **regeneration buffer**, mix by gently shaking the tube for 5 sec; centrifuge and discard the supernatant. Repeat twice. Continue with **step B.2**

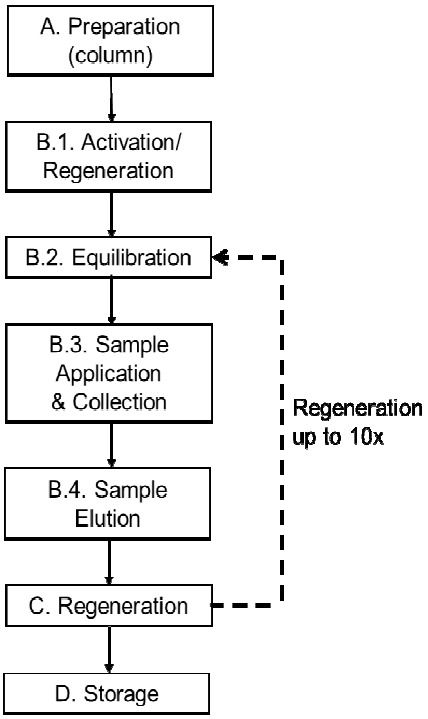
D. Storage

Resuspend the EndoTrap® HD gel pellet in 1 gel volume of **storage buffer**, supplemented with **2.5 ppm ProClin™ or 0.02% sodium azide** and store at 2-8 °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.

[1] The regeneration substance is NOT (sodium) deoxycholate! DOC would have cytotoxical effects on cell culture and also influence the cell growth and the morphology of the cells. It is reported that DOC induces DNA damage.
[2] See page 12: 3.2 Custom Specific Equilibration Buffer

2.2 Protocol Column Mode for EndoTrap® HD 1/1 and 5/1

A. Preparation



To use a **prepacked column,** place the column in a suitable holder and first remove the **top cap**. This prevents air bubbles from emerging. Next remove **bottom cap**. Allow the storage solution to drain from the column [~ 8 min]. The flow stops automatically when the solution reaches the upper disc. 5x buffers must be diluted 1:5 with endotoxin-free water prior to use.

B. Activation and Endotoxin Removal

1.Fill the column with 3 mL **regeneration buffer**♦ and let the column drains out completely. Repeat this step [~12 min].

2. Fill the column with 3 mL **equilibration buffer** or customer specific buffer and let the column drain out completely. Repeat this step [~12 min].

3. Apply your **sample** (either **in equilibration buffer** or in customer specific buffer) onto the column and start collecting the fractions (depending on the applied sample volume) immediately. The applied sample elutes directly after the column void volume (0.3-0.5 mL). The column can be constantly filled up, until the whole sample (up to 50 mL) has been applied. Afterwards let the sample drain completely from column [flow rate: 0.2 to 1 mL/min]. 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.

4. In order to elute your entire sample, apply extra 1 mL **equilibration buffer** or customer specific buffer, let the column drain out and collect the flow through completely. [As substances pass through the column at different rates, it is important to test each fraction for the sample concentration. This can be done by measuring the UV-absorption of the flow through fractions.]

C. Regeneration

Fill the column with 3 mL **regeneration buffer** or customer specific buffer and let the column drain out completely. Repeat once [~ 12 min]. **Continue with step B.2**

D. Storage

Apply 1 mL **storage buffer** supplement with **2.5 ppm ProClin™ or 0.02% sodium azide** and let the column drain off completely. Close the bottom cap of the column and apply 1 mL storage buffer, supplement with **2.5 ppm ProClin™ or 0.02% sodium azide**, close the column 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.

♦ The regeneration substance is NOT (sodium) deoxycholate! DOC would have cytotoxical effects on cell culture and also influence the cell growth and the morphology of the cells. It is reported that DOC induces DNA damage.

2.3 Operating EndoTrap® HD on Large Scale

Column dimension[1]

The column dimension to be applied in a process depends largely on sample composition and volume. The following constraints have to be considered:

1. Endotoxin content of the sample
2. Time-on-the-column (minimum time required for exchange)
3. Volume to be processed (flow rate)

Endotoxin content of the sample

- For optimal results, total LPS units applied should not exceed 30-50% of the maximum column capacity (5 x 10^6 EU/mL resin).

Time-on-the-column

- Time-on-the-column should be minimum 30 seconds.
  Example: A 10 mL column should be processed with a maximum flow rate of 20 mL/min.
  Please note that this is not the optimal flow rate for every sample.

Volume to be processed

- In order to process a certain volume in a certain time, the column dimension (diameter vs. length) must allow a reasonable flow rate
- The flow rate could be in the range of 60 to 840 cm/h
- The column size ratio should be between 1:1 and 1:3 (diameter:length)

- 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**

Packing procedure	Use equilibration buffer for packing the column. Packing velocity should be in the range of 800 to 1000 cm/h. The operating pressure should not exceed 0.3 MPa (43.5 psi).
Equilibration	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.* $CaCl_2$). Protocol: Pre-equilibrate the column with 3 column volumes equilibration buffer plus 1 M NaCl Equilibrate the column with 3 column volumes equilibration buffer. Max. flow rate: 600 to 840 cm/h
Endotoxin removal	EndoTrap® HD works under a broad range of conditions, there are nearly no limits regarding pH, ionic strength and additives. Sample buffer: Customer defined Volume: Customer defined Max. flow rate: 600 to 840 cm/h
Regeneration	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 Max. flow rate: 600 to 840 cm/h

1 For examples see table 1 "Column dimension" on page 12.

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 (stable up to 3 months at room temperature)
	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	

2.4 Optional Steps (Column / Batch Mode)

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 Column Dimension

Table 1: Column dimension: We recommend a column size ratio between 1:1 and 1:3 (diameter:length). The maximum flow rate should not exceed 1000 cm/h (please note that this is not the optimal flow rate for every sample).

Resin volume	**10 mL**	**50 mL**	**250 mL**	**250 mL**
Column dimension	1.6 cm x 5 cm	3 cm x 7 cm	5 cm x 12 cm	6 cm x 9 cm
Max. flow rate [mL/min]	20 mL/min	100 mL/min	275 mL/min	396 mL/min
Max. flow rate [cm/h]	600 cm/h	840 cm/h	840 cm/h	840 cm/h
Time-on-the-column (Max. flow)	0.5 min	0.5 min	0.9 min	0.6 min
Process volume	1.2 litre/h	6 litre/h	16.5 litre/h	23.8 litre/h

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.: Ca^{2+} (*e.g.* $CaCl_2$) has to be added freshly to **your** customer specific buffer	0.1-10 mM Ca^{2+}
	Substitute for Calcium: Mg^{2+} (e.g. $MgCl_2$) has to be added freshly to your customer specific buffer	0.1-10 mM Mg^{2+}
	Possible additives:	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 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, bacteriophages
	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

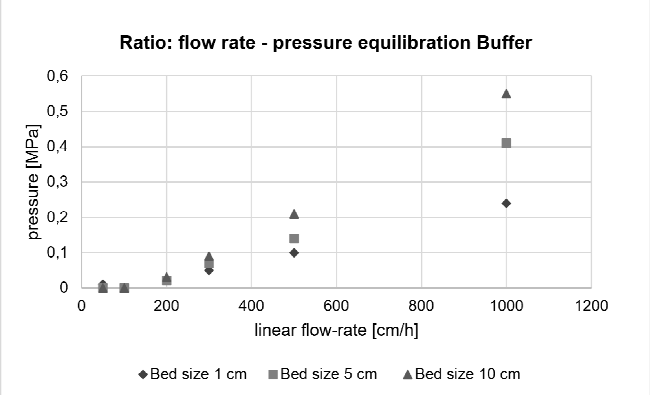
Table 5: Sanitisation test: Batch mode: Endotoxin removal of 1.5 mL endotoxin spiked BSA (20 mg/mL, 600 EU/mL) with 0.1 mL EndoTrap® HD resin. The indicated sanitisation buffer provides 100% reduction of bacterial contamination (10^7 CFU incubated for indicated time). Endotoxin removal is not affected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

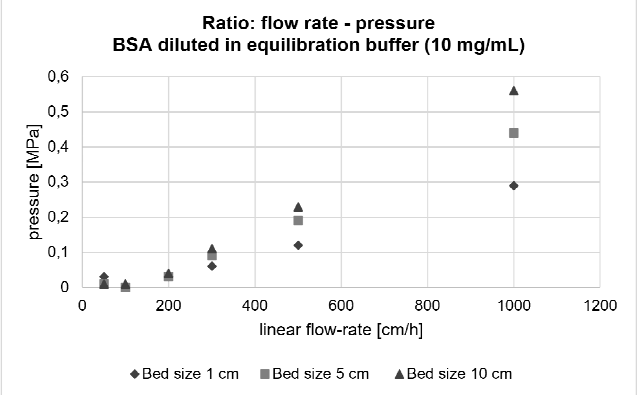
Sanitisation buffer	Incubation time	Endotoxin removal efficiency [%]	Factor of reduction [CFU]	
			Listeria	*E. coli*
0.1 M Acetic acid + 20% EtOH	4 hours	99.89	10^7	10^7
70% EtOH	6 hours	99.82	10^7	10^7
0.1 M HCl	6 hours	99.87	10^7	10^7

3.6 Pressure / Flow Comparison

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(TM). EndoTrap® HD Storage Buffer has to be supplemented with 2.5 ppm ProClin(TM) 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 LIONEX GmbH:

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

For more information or technical assistance, call, write, fax or e-mail.

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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
- **EndoGrade® Water** – Endotoxin concentration < 0.001 EU/mL

EndoTrap® red

- **EndoTrap®** red - especially for the use with buffers containing calcium chelators

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EndoTrap® HD patented technology has been exclusively licensed to LIONEX GmbH and is provided for research and biomanufacturing use only.