Index

1 Purpose of the Document

Purpose	Production of products in a regulated environment requires detailed documentation of all components used. The purpose of the Regulatory Support File (RSF) is to provide comprehensive information about EndoTrap HD affinity matrix to help customers register their production processes. Information described here may also be useful for quality control and setting up procedures for cleaning-in-place (CIP) and sanitization.

2 Quality policy

LIONEX GmbH	It is of highest interest for all LIONEX employees to continuously improve the satisfaction of our customers and all business partners and permanently fulfil their needs and desires to establish successful enduring partnerships. This will be achieved by: - Development and manufacturing of innovative products of outstanding quality - Provision of extensive customer support and service - Well-educated, highly dedicated employees - Maintenance and continuous improvement of our quality management system - Compliance with all relevant statutory requirements
Bead manufacturer	Tosoh Bioscience GmbH, the manufacturer of our beads, is certificated according to ISO 9001.
Selection of reagent supplier	LIONEX selects all its' reagent suppliers appropriate to defined quality aspects. One of our criteria is the supplier's certification according to standards that are required for our products or processes. The fulfilment of our demands is monitored regularly by early supplier assessments or audits.

2.1 Certifications

ISO standards	Since October 2009 LIONEX is certified according DIN EN ISO 13485:2016.
Regulatory requirements	There are no regulatory requirements (such as GMP) for the production of EndoTrap HD.

2.2 Certificate for Quality Assurance

LIONEX GmbH	DIN EN ISO 13485:2016. Scope: Development, manufacturing and distribution of biotechnological products for use in the sectors of life science, food analysis and human diagnostics.
Tosoh Corporation Tosoh Techno-System Inc. Tosoh Hi-Tec, Inc. Tosoh AIA, Inc.	ISO 9001:2015, JIS Q 14001:2015 Scope: Design, manufacturing, sales and services of in vitro diagnostic medical devices, genetic analysis devices, liquid chromatography systems and separation media.
Tosoh Bioscience, LLC	QSR®´s* Contract and ISO 9001:2015 Scope: Supplying bio-separation products to the pharmaceutical and biotechnology industries. Exclusions: 7.3 Design and development; 7.5.1 Control of production and service provision (service provision only); 7.5.2 Validation of processes for production and service provision; 7.5.4 Customer property.
Tosoh Bioscience GmbH	ISO 9001:2015 Field of application: development and distribution of HPLC-columns and separation media for purification and production of bio-products

* QSR: Quality System Regulation
♠ ISO: International Organization for Standardization

3 Product description

Content of Chapter 3	Chapter 3 provides a general overview on the basic features of EndoTrap HD affinity matrix and endotoxin analysis - What is EndoTrap HD - General product features - Endotoxin (definitions, characteristics, detection methods, unit definition)

3.1 What is EndoTrap® HD

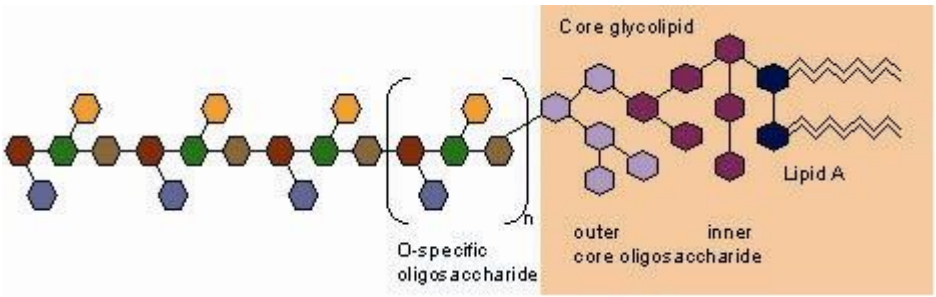
General description	- EndoTrap HD is an affinity matrix suited for removal of endotoxin. It consists of a phage-derived protein ligand covalently bound to an acrylate bead matrix. The ligand recognizes the conserved inner core of lipopolysaccharides (LPS). High affinity binding requires low concentration of calcium ions.

3.2 General Product Features

Intended use of the product	Removal of bacterial lipopolysaccharides from biological solutions.
Principle of the product	Affinity chromatography
Origin of protein ligand	Bacteriophage tail protein
Ligand specificity	Inner core region of LPS of gram-negative bacteria.
Requirement for LPS binding	at least 0.1 mM free calcium ions, e.g. supplemented as $CaCl_2$
Amount of immobilized protein	approx. 1 - 3 mg/ml

3.3 Endotoxin (definitions & characteristics)

Definition pyrogen	Any substance which would induce a temperature rise when introduced into an organism. There are a lot of substances that can cause fever in mammals; examples are bacterial DNA, LTA (from Gram-positive bacteria), porins or superantigens.[1]
Definition endotoxin	The term "endotoxin" is occasionally used to refer to any cell-associated bacterial toxin, in bacteriology it is properly reserved to refer to the lipopolysaccharide complex associated with the outer membrane of Gram-negative pathogens.[2]
Definition LPS	Lipopolysaccharide; major component of the outer membrane of Gram-negative bacteria.
Structure of LPS	Large molecule consisting of a lipid and a polysaccharide (carbohydrate) joined by a covalent bond. The LPS consists of three general parts: - Variable region called **o-specific chain** - Relatively conserved **core region** - Conserved **Lipid A**



"Inner core region" of LPS	Consisting of heptoses and keto-3-deoxy-octonic acid (KDO) forming a rare sugar-motive which is highly conserved.
Physical characteristics of LPS[1]	- complex amphipathic molecules - net negative charge tendency - molecular weight: 10 – 20 kDa - isoelectric point (pI): ~ 2.0 - critical micelle concentration (CMC): ~ 4 µM

3.4 Endotoxin (detection methods & unit definition)

[1] Endotoxin Compendium, V 10.13, Hyglos GmbH
[2] http://www.textbookofbacteriology.net/endotoxin.html

Detection methods for LPS	- EndoLISA® endotoxin assay: *sensitivity: 0.05 – 500 EU/ml* - EndoZyme® endotoxin assay: *sensitivity: 0.005 – 500 EU/ml* - Recombinant Factor C assay: *sensitivity: 0.01 – 10 EU/ml* - Rabbit pyrogen test (Ph.Eur. 2002, 2.6.8) *sensitivity: "13.81 EU/mL/kg as the concentration of endotoxin necessary to induce a temperature rise of 0.5°C"*[1] - **Limulus amebocyte lysate test**[2] (Ph.Eur. 2002, 2.6.14) Gel Clot LAL: *sensitivity: 0.03 – 0.25 EU/ml* Chromogenic End-Point LAL: *sensitivity: 0.1 – 1 EU/ml* Kinetic Turbidimetric LAL: *sensitivity: 0.01 – 100 EU/ml* Kinetic Chromogenic LAL: *sensitivity: 0.005 – 50 EU/ml* - NIH-3T3 fibroblasts activation-cytokine assay *sensitivity: 10-20 pg/ml = 0.1-0.2 EU/ml*[3]

[1] Rosimar L. Silveira, et al. Comparative evaluation of pyrogens tests in pharmaceutical products. Brazilian Journal of Microbiology (2004) 35: 48-53.
[2] Endotoxin Compendium, V 2.6, Hyglos GmbH
[3] Fraunhofer Institut für Grenzflächen- und Bioverfahrenstechnik, Jahresbericht 2006/2007, page 62

Endotoxin unit definition	Endotoxin unit (EU) describes the biological activity of an endotoxin molecule. The **biological activity** causing pyrogenic effects depends on a variety of factors like polysaccharide chain length, aggregation status, solubility in biological fluids, bacterial source, associated substances, etc.[1]
	1 EU corresponds to ~100 pg of endotoxin. 1 EU = 1 IU (common standard for the United States Pharmacopoeia, the World Health Organization and the European Pharmacopoeia)

[1] Endotoxin Compendium, V 2.6, Hyglos GmbH

4 Specifications

LPS Binding Ligand	EndoTrap® HD ligand	
	Protein structure:	homo-trimer
	Molekular weight:	150 kDa (trimer)
	Binding constant:	KD = 5 x 10^{-8} M
	Isoelectric point:	8.52

Bead 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	Immobilized ligand:	approx. 1 - 3 mg/ml
	Binding capacity:	> 5 x 10^{6} EU/ml resin
	Operating pH range:	pH 4 - 10
	Operating flow rate:	maximum 600 – 840 cm/h
	Operating pressure:	up to 0.3 MPa is recommended (maximum pressure drop on column is 0.7 MPa)
	Temperature stability:	4 – 35 °C
	Ligand leakage:	< 20 ng/ml
	Shipping condition:	ambient temperature

5 Operating, Column dimension & Applications

Content of Chapter 5

Chapter 5 provides a detailed operating guidance and application data referring to the **Package Insert**:

- Operating
- Column dimension
- Equilibration buffer
- Samples to be applied
- Tested LPS sources
- Applications data:
  pH
  Ionic strength
  Sanitisation buffers
  Applied samples: BSA, IgG, Lysozym
  Pressure / flow comparison
  Reusibility

6 Product stability

A) **Chemical stability**	Discusses all leakage studies performed, including detection and quantification of extractable compounds. There is also information on shelf life and storage stability.
B) **Chromatographic stability**	Is deduced from the results of the stability studies on the chromatographic medium, i.e. the elution pattern behaviour and the pressure/flow rate changes after treatment with different physical conditions and chemical agents.

A) Chemical stability

6.1 LPS-binding ligand

Please see chapter 7.1.

6.2 Bead resin

Please see chapter 7.2.

6.3 Matrix (EndoTrap HD)

Please see chapter 7.3.

6.4 Protease

Protease as sample	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. Example: When using pepsin, work above pH 6 since pepsin is an acidic protease..

6.5 Storage

Storage	Unused resin can be stored in the container. Ensure that the container is densely closed. EndoTrap HD is delivered in 20 mM sodium phosphate 150 mM NaCl, 2 mM EDTA, pH 7.4, 2.5 ppm ProClin™
	Fresh material: at 2-8 °C as supplied
	Regenerated material: at 2-8 °C in storage buffer supplemented with 2.5 ppm ProClin™ or 0.02% sodium azide
	Alternatively 20% ethanol can be used as storage buffer; the storage time will then be reduced to 4 weeks.
	Note: **Do not freeze!**
	HD resin is stable up to 3 month at room temperature

6.6 Shelf life

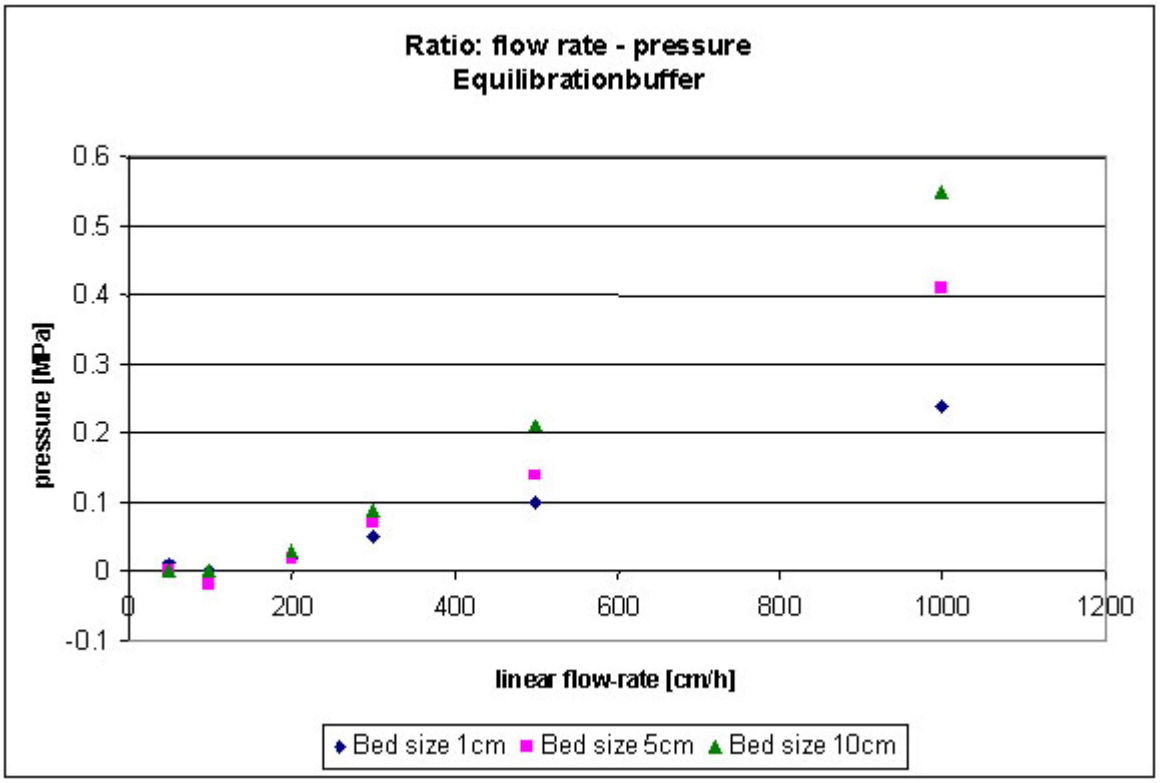
Shelf life	24 month for unused material when stored at 2-8 °C. Unused material is stable until the stated expiry date when stored correctly (at 2-8°C).

B) Chromatographic stability

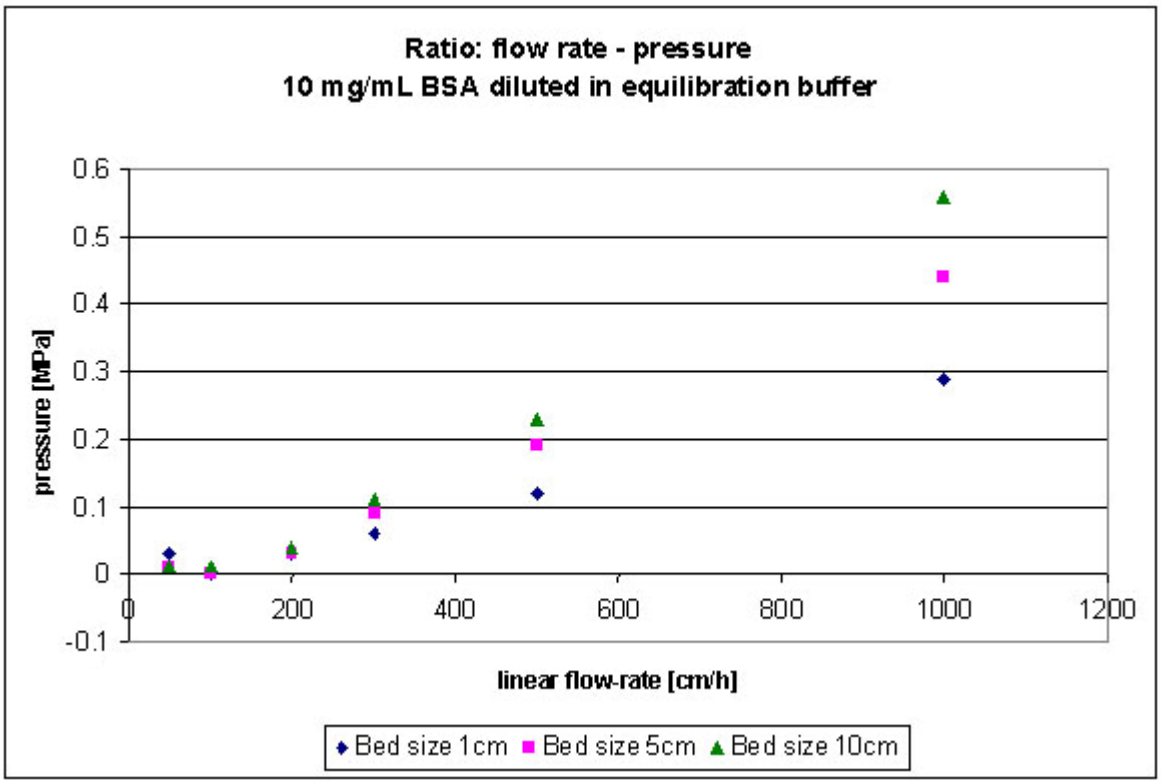
6.7 Pressure / flow comparison

	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

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.



Equilibration buffer



BSA solution [10 mg/ml]

6.8 Elution pattern behaviour

Please look into chapter 8.

6.9 Regeneration

Regeneration	EndoTrap HD can be regenerated under mild conditions by complexing Ca^{2+} with EDTA at elevated ionic strength.
	Regeneration buffer: 20 mM Hepes, 1 M NaCl, 2 mM EDTA, pH 7.5
	Protocol: Clean the column with 6 column volumes regeneration buffer.
	Flow rate: 600 – 840 cm/hr

6.10 Re-usability

Cycle number	Endotoxin removal efficiency [%]	Sample Recovery BSA [%]
0	99.9955641	102.5
1	99.9946041	99.5
2	99.9948371	97.5
3	99.9948335	99.5
4	99.9946371	96
5	99.9948401	97
6	99.9944963	97
7	99.9949815	97
8	99.9913897	96.5
9	99.9945602	96
10	99.9955641	102.5

Re-usability: 100 ml BSA solution (10 mg/ml) was spiked with 10^6 endotoxin units and endotoxin removal by EndoTrap HD (10 ml column) was measured. After each removal step the resin was regenerated with regeneration buffer and equilibrated with Hepes buffer (20 mM Hepes, pH 7.5; 150mM NaCl, 0.1 mM $CaCl_2$) before starting the next cycle.

6.11 Cleaning in place (CIP)

Cleaning in place	CIP should remove 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.
	Flow rate: 600 – 840 cm/hr

6.12 Sanitisation

Sanitisation	Sanitisation reduces microbial contamination of the resin to a minimum.
	Recommended sanitisation buffer: 0.1 M Acetic acid + 20% Ethanol
	Protocol: Incubate the column with sanitisation buffer for 2 – 12 hours.

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

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 provided 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.

7 Analytical methods

Protocols of listed analytical methods are available on request.

LPS-binding ligand	- LPS concentration - Microbial contamination - Purity of the ligand
Bead resin	- particle size distribution - coupled protein amount
Matrix (EndoTrap HD)	- LPS binding capacity - LPS removal efficiency - Microbial contamination - Ligand leakage

7.1 Analytical methods for the LPS-binding ligand

Specifications / Characteristics	**Limit**	**Analytical method**
LPS concentration	< 15 EU/mg	EndoZyme® recombinant Factor C (rFC) endotoxin detection assay LAL assay “Kinetic Chromogenic LAL assay”
Microbial contamination (microorganism / ml suspension)	< 10 cfu/ml	Bacterial growth on plates
Purity of the LPS-binding protein (ligand)	> 97.5% no aggregates visibly no foreign protein visible	Gel filtration UV spectrum SDS-PAGE Fluorescence spectrum (310-330 nm)
Concentration of the LPS-binding protein (ligand)		UV spectrum

7.2 Analytical methods for the bead resin

Specifications / Characteristics	**Limit**	**Analytical method**
Particle size distribution[1]	> 80% of the particles must be between 40-90 µm	It is measured using a Coulter Counter according to manufacturer´s protocol (Coulter Electronics).
Exclusion limit[2]	♣	♣
Microbial contamination[2]	♣	♣
LPS concentration[2]	♣	♣
Eluable matter[2]	♣	♣
Colored particles and other foreign substances[2]	♣	♣

QC Methods[2] Tosoh Bioscience	All Toyopearl resins are tested before release. All Tosoh Bioscience resins are inspected for the presence of any foreign material before release.

♣ Please contact Tosoh Bioscience for more detailed information.

[1] Toyopearl® AffiPak™ ACT LabPak Sampler, Tosoh Bioscience
[2] Drug Master File for Toyopearl® Chromatographic Resins, File Number BB-MF-3907, February 2006, Tosoh Bioscience LLC

7.3 Analytical methods for matrix

Specifications / Characteristics	**Limit**	**Analytical method**
Coupled protein amount	≥ 4 mg/ml beads	Indirectly via A280, measurement of the ligand concentration difference before and after coupling
LPS binding capacity	≥ 5 x 10^6 EU/ml resin 1.5 ml BSA [10 mg/ml] on 0.3 mL resin, spiked with 4.5 x 10^6 EU LPS)	Indirect via LAL assay (before / after using EndoTrap HD)
LPS removal efficiency	≥ 99% (from 1.5 ml BSA [10 mg/ml]] on 0.3 mL resin, spiked with 7.5 x 10^3 EU LPS)	Indirect via LAL assay (before / after using EndoTrap HD)
Microbial contamination	< 10 cfu/ml	Bacterial growth on plates
Ligand leakage	< 20 ng/mg	Leakage ELISA, according to LIONEX protocol

8 Extractable compounds

8.1 Extractable compounds from bead resin (Toyopearl)[1]

An activated bead matrix is used for the coupling of EndoTrap ligand. Leakage of residual monomers and reactants from EndoTrap HD has not been tested. Please contact Tosoh Bioscience for more detailed information.

8.2 Extractable compounds from manufacturing process

Raw materials used in the manufacture of EndoTrap HD, which may give rise to potential contaminants are shown below:

Extractable compounds	**Analytical methods**
Boric acid, EDTA, GdnHCl, Citrate, Ethanolamin, NaCl	A chromogenic assay for EDTA has been reported. (Sorensen, K., An easy microtiter plate-based chromogenic assay for ethylenediaminetetraacetic acid and similar chelating agents in biochemical samples. Anal. Biochem., 206(1), 210-211 (1992))

[1] Drug Master File for Toyopearl® Chromatographic Resins, File Number BB-MF-3907, February 2006, Tosoh Bioscience LLC

8.3 Extractable compounds from EndoTrap HD

To ensure low ligand leakage we recommend starting the protocol with a regeneration step followed by an equilibration step, therefore the concentration of leached ligand in fractions should be in the range of 300 pg/ml to 10 ng/ml.

Our experiments showed that 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 your sample we recommend collecting the first column volume separately.

When applying concentrated sample solutions (e.g. > 5 mg/ml) the concentration of leached ligand could be up to 20 ng/ml in the very first fraction.

Extractable compounds	**Amount**	**Analytical methods**
LPS-binding ligand	< 20 ng/ml	EndoTrap Leakage ELISA, according to LIONEX protocol

Extractable compounds from bead resin

(please see 8.1)

8.4 Extractable compounds from storage buffers

Raw materials used in the manufacture of EndoTrap HD, which may give rise to potential contaminants are shown below:

Extractable compounds	**Amount**	**Analytical methods**
ProClin™ 150	2.5 ppm	Literature from Sigma-Aldrich available:
<u>Active Ingredients:</u>	<u>CAS-numbers:</u>	
- 5-chloro-2-methyl-4-isothiazolin-3-one	26172-55-4	Analytical Technique - Chemical Standards: *ProClin 150 Preservative for Diagnostic Reagents*
- 2-methyl-4-isothiazolin-3-one	2682-20-4	
- magnesium chloride	14989-29-8	
- magnesium nitrate	10377-60-3	
<u>Others:</u> Tris, NaCl, EDTA		For EDTA please see 8.2

9 EndoTrap Leakage ELISA

Introduction	Depending on the intended use of the preparation and the step in the purification (early or late), where EndoTrap is used, a quantitative analysis of residual EndoTrap ligand might be required. The EndoTrap Leakage ELISA has been developed to allow an accurate and reproducible determination of small amounts of EndoTrap ligand in biological samples. This ELISA is suitable for detection of leached ligand from EndoTrap HD resin.

9.1 Specifications of EndoTrap Leakage ELISA

Intended use	Quantification of EndoTrap HD ligand leakage in biological aqueous solutions.
Secificity	A specific monoclonal antibody to EndoTrap ligand (EndoTrap HD) are used in the assay. Cross-reaction with other proteins is not known. This ELISA is **not** suitable to detect leached EndoTrap red ligand.
Measuring range	2000 pg/ml to 30 pg/ml
Limit of Quantification (LOQ)	30 pg/ml EndoTrap ligand
Storage	Store at 2-8°C Stable up to 1 month at room temperature

9.2 Package Insert EndoTrap Leakage ELISA

Package insert including detailed operating guidance is enclosed on the following pages.

10 Toxicological data

10.1 Toxicological data – EndoTrap ligand

The EndoTrap ligand is a protein by nature, which is bound to the polymeric bead-matrix by stable covalent bonds. However, leakage of minute amounts of ligand is a matter of fact for all affinity materials. Depending on the intended use of the preparation and the step in the purification (early or late), where EndoTrap is used, different amount of EndoTrap ligand will leached into the sample pool.

To make a statement regarding the immunomodulatory and toxicological properties of the EndoTrap ligand several in-vitro studies were performed:

Immunomodulatory properties in cultures of murine splenocytes	Study design: Determination of the immunostimulatory properties of EndoTrap HD ligand in the murine splenocyte model system. In this assay system murine splenocytes/ml were stimulated with 20 µl (corresponding to 20 µg antigen) of EndoTrap HD ligand. Indicated cytokines were determined from the precleared supernatants using commercial kits.
	Conclusion: These experiments clearly demonstrate, that under the reported conditions EndoTrap HD antigen revealed no detectable pyrogenic and mitogenic properties to activate the innate immune system. [Report LO-060005 attached.]
Tests for cytotoxicity: *in vitro*-methods DIN ISO 10993-5 Testung der biologischen Verträglichkeit / Biokompatibilität nach DIN ISO 10993-5	Study design: Determination of the cytotoxicity of EndoTrap HD ligand in the murine fibroblast cell line L929 according to ISO 10993-5. Following criteria were addressed: Influence of EndoTrap HD ligand on - the cell vitality (fluorescein-diacetate (FDA) / propidium iodide staining (PI)) - mitosis activity (fluorescein-diacetate / propidium iodide staining) - cell density and cell spread (fluorescein-diacetate / propidium iodide staining) - cell morphology / cell spread (Hemalaun stainig) - metabolic activity (quantitation of cellular protein using a Bradford assay) - DNA synthesis (3H thymidine incorporation)

Conclusion:
These experiments clearly demonstrate, that under the reported conditions EndoTrap HD antigens revealed no detectable cytotoxic effects in cultures of murine L929 fibroblasts and splenic cells.

[Report LO-060006 attached.]

10.2 Toxicological data – bead matrix

Please contact Tosoh Bioscience for more detailed information[1].

[1] Drug Master File for Toyopearl® Chromatographic Resins, File Number BB-MF-3907, February 2006, Tosoh Bioscience LLC

10.3 Toxicological data – Raw material

Substance	**Supplier** **Catalogue number** **CAS-numbers**	**Comment**
Storage buffer: ProClin™ 150	Supelco (Sigma-Aldrich) Cat. no. 49376-U	Please use TOXNET or HSDB for detailed information.
<u>Active Ingredients:</u>	<u>CAS-numbers:</u>	<u>Hazards (risk):</u>
- 5-chloro-2-methyl-4-isothiazolin-3-one	26172-55-4	R23/24/25, R34, R43, R50/53
- 2-methyl-4-isothiazolin-3-one	2682-20-4	
- magnesium chloride	14989-29-8	
- magnesium nitrate	10377-60-3	R8, R36
Residual monomers and reactants from bead matrix:	Please contact Tosoh Bioscience for more detailed information.	

TOXNET - Databases on toxicology, hazardous chemicals, environmental health, and toxic releases:
http://toxnet.nlm.nih.gov/

Hazardous Substances Data Bank – HSDB:
http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

11 Application Examples

11.1 Influence of buffer additives

Buffer additives	**Endotoxin removal efficiency [%]**	**“Suitable”**
Imidazol, 50 mM	99.989	Yes
Imidazol, 300 mM	99.989	Yes
Glycerin, 2%	99.994	Yes
Glycerin, 10%	99.994	Yes
Glycerin, 20%	99.979	Yes
Urea, 0.5 M	99.993	Yes
Urea, 1 M	99.990	Yes
Urea, 1.5 M	99.992	Yes
DTT, 1 mM	99.991	Yes
DTT, 10 mM	99.977	Yes
DTT, 15 mM	99.957	Yes
Tween20, 0.005%	99.992	Yes
Tween20, 0.05%	99.994	Yes
Tween20, 0.5%	99.995	Yes
EtOH, 10%	99.990	Yes
EtOH, 20%	99.983	Yes
Arginin, 10 mM	99.985	Yes
Arginin, 100 mM	99.952	No
Arginin, 300 mM	99.927	No

Influence of buffer additives on endotoxin removal efficiency: Batch mode (200 µl resin): 1.5 ml endotoxin spiked BSA (10 mg/ml, 10,000 EU/ml) was incubated in HEPES buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 0.1 mM $CaCl_2$) with the indicated additives for 60 minutes and endotoxin removal by EndoTrap HD was measured.

Positive control: HEPES buffer spiked with BSA (10 mg/ml, 10,000 EU/ml) without additives.

12 Supplementary Information

12.1 CoA – Certificate of Analysis

End-product: EndoTrap® HD

CoA´s is enclosed on the following pages.

12.2 MSDS – Material Safety Data Sheet

MSDS – Europe	EndoTrap HD
MSDS – US	EndoTrap HD

MSDS´s are enclosed on the following pages.

12.3 Drug Master File – Toyopearl

DMF: Toyopearl Affinity Resin Toyopearl Epoxy Resin	Please contact Tosoh Bioscience for any information considering the "Drug Master File": Toyopearl® Affinity Resin or any other question regarding Toyopearl® Epoxy Affinity Resin. **Europe:** Tosoh Bioscience GmbH Zettachring 6 DE-70567 Stuttgart www.tosohbioscience.de **United States:** Tosoh Bioscience LLC 156 Keystone Drive USA - Montgomeryville, PA 18936-9637 www.tosohbioscience.com

12.4 List of Trademarks

EndoTrap®	is a registered international trademark of Hyglos GmbH exclusively licensed to LIONEX GmbH, Germany.
EndoLISA®	is a registered international trademark of Hyglos GmbH, Germany.
EndoZyme®	is a registered international trademark of Hyglos Invest GmbH, Germany.
TOYOPEARL®	is registered trademark of Tosoh Corporation, the parent company of Tosoh Bioscience.
ProClin™	is a registered trademark of Rohm and Haas Company.
Tween20®	is a registered trademark of ICI America, Inc.

12.5 Customer publications and reference list

For **EndoTrap® product information** please contact

LIONEX GmbH, Salzdahlumer Strasse 196, 38126 Braunschweig, Germany

@ info@lionex.de +49 531 260 12 66

For orders: sales@lionex.de

2019

269 Hietala, Ville et.al.: **The Removal of Endo- and Enterotoxins From Bacteriophage Preparations** Front. Microbiol., 23 July 2019 https://doi.org/10.3389/fmicb.2019.01674

2015

268 Robert, Leah**: Bacteriophages for use against bacterial infections**, www.freepatentsonline.com/y2015/0247127.html

267 Rolando, Monica et. al.**: Contractile actin cables induced by Bacillus anthracis lethal toxin depend on the histone acetylation machinery** *Cytoskeleton*. onlinelibrary.wiley.com/doi/10.1002/cm.21256/abstract

266 Guillou, Clément et. al.**: Prophylactic Injection of Recombinant Alpha-Enolase Reduces Arthritis Severity in the Collagen-Induced Arthritis Mice Model** *PLoS ONE 10* Issue *8*, P. e0136359. journals.plos.org/plosone/article?id=10.1371/journal.pone.0136359

265 Majewska, Joanna et. al.**: Oral Application of T4 Phage Induces Weak Antibody Production in the Gut and in the Blood** *Viruses 7* Issue *8*, P. 4783–4799. www.mdpi.com/1999-4915/7/8/2845

264 Saunders, Kevin O. et. al.**: Broadly Neutralizing Human Immunodeficiency Virus Type 1 Antibody Gene Transfer Protects Nonhuman Primates from Mucosal Simian-Human Immunodeficiency Virus Infection** *Journal of Virology 89* Issue *16*, P. 8334–8345. https://jvi.asm.org/content/89/16/8334.full

263 Ji, Jing et. al.**: The B subunit of Escherichia coli heat-labile toxin alters the development and antigen-presenting capacity of dendritic cells** *Journal of Cellular and Molecular Medicine 19* Issue *8*, P. 2019–2031. onlinelibrary.wiley.com/doi/10.1111/jcmm.12599/abstract

262 Devera, T. Scott et. al.**: Immunization of Mice with Anthrax Protective Antigen Limits Cardiotoxicity but Not Hepatotoxicity Following Lethal Toxin Challenge** *Toxins 7* Issue *7*, P. 2371–2384. www.mdpi.com/2072-6651/7/7/2371

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