EndoTrap®
Endotoxin Removal System
Chromatography resin for endotoxin removal
in biomanufacturing processes

LIONEX Diagnostics and Therapeutics

Application Protocol for Pilot Scale EndoTrap®

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Content

How to calculate the number of required cleaning steps?

EndoTrap can be reused at least three times (EndoTrap **HD** up to 10 times) without any loss of endotoxin removal efficiency. In case your starting endotoxin concentration is very high or in case you wish to reach a very low concentration, EndoTrap can be applied in a consecutive manner several times. Each round of application theoretically yields a two log reduction of endotoxin.
However, parameters such as pH, ionic strength, temperature, contact time, etc. might have to be optimized for each application to obtain maximum endotoxin removal with minimum loss of product.
Depending on your LPS starting concentration [EU] you must perform a certain number of cleaning steps, in order to achieve your desired LPS end concentration [EU].

Starting LPS concentration (buffer) [EU]	**After 1. cleaning step [EU]**	**regeneration step** (kit includes regeneration buffer)	**After 2. cleaning step [EU]**	**regeneration step** (kit includes regeneration buffer)	**After 3. cleaning step [EU]**
1.000.000	10.000		100		1
100.000	1.000		10		0.1
10.000	100		1		0.01
1.000	10		0.1		<0.005
100	1		0.01		<0.005
10	0.1		<0.005		
1	0.01		<0.005		
0.1	<0.005				

LPS removal from **buffers**: With repetitive use of EndoTrap, you can achieve concentrations as low as 0.005 EU/ml.

LPS removal from **proteins**: With repetitive use of EndoTrap, decrease to concentrations as low as 0.1 EU/ml is possible. As it is a biological system, the efficiency of EndoTrap slightly decreases at low endotoxin contamination levels. At 0.1 EU/ml the removal efficiency is approximately 70%.

If you do not yield your desired endotoxin level after the third cleaning step (each cleaning step finished with a regeneration step), please contact us and use our technical support!

Protocols

Chromatography is traditionally done in two modes: discontinuous (batch mode) and continuous (column mode) chromatography. EndoTrap can be used either in batch or column mode. In general endotoxin 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, contact time etc. might have to be optimized for each application to obtain maximum endotoxin removal with minimum loss of product.**

Precautions

- EndoTrap® HD resin and columns are supplied with ProClin™ as preservative. For further information see the EndoTrap® HD Material Safety Data Sheet.All used materials like containers or pipette tips and buffers must be endotoxin free. Glass ware is preferred, as endotoxins can be removed by heat treatment (200°C, 4 h or 250°C, 1 h).
- Buffers should be prepared from endotoxin free materials and endotoxin free water.
- Buffers, resin and sample should have the same temperature (4-20°C) during the cleaning steps.
- When using EndoTrap® columns, all buffers including equilibration buffer and regeneration buffer should be **degassed** prior to use. When using EndoTrap® gel slurry, degas slurry prior to use.
- EndoTrap® 5x buffers have to be 1:10 diluted with endotoxin free water.
- If you use your customer specific buffers and EndoTrap® HD: Buffers used for endotoxin removal with EndoTrap® HD need to contain 50-100 µM Ca^{2+}.

Protocol performed on fully automated liquid chromatography systems

HPLC/FPLC column preparation

- Rinse the empty HPLC/FPLC column thoroughly with endotoxin-free water. Store over night in 1 M NaOH to ensure no contamination with endotoxins. Afterwards wash the column with endotoxin-free water to remove residuals of NaOH completely.

Preparation of HPLC/FPLC system

- Rinse the whole HPLC/FPLC system thoroughly with endotoxin-free water and 1 M NaOH. Keep in 1 M NaOH over night to ensure no contamination with endotoxins. Afterwards wash the system with endotoxin-free water to remove residuals of NaOH completely.

Preparation of EndoTrap® column

1. Assemble the bottom part of the HPLC/FPLC column as described in the manufacturers' manual.
2. Leave the colums' top open. Rinse with endotoxin-free water. Seal the outlet at the bottom after rinsing.
3. Resuspend the EndoTrap slurry, so that no sediment is left.
4. Fill the column with EndoTrap slurry. For this you can use a endotoxin-free funnel.
5. Make sure that the column is closed at the bottom, to prevent the buffer from running out.
6. Cover the top of the column with endotoxin-free Parafilm or aluminium foil. For example use aluminium foil that has been heated to 200°C for 3h.
7. When the resin is settled, open the outlet to drain the buffer, but do not let the resin run dry. Do not drain the buffer before the resin is completely settled, because air bubbles may form in the resin.
8. Repeat the filling steps until all of the resin is in the column.

- Assemble the top part of the HPLC/FPLC column following the manufactures' manual.
- The column can be used following the EndoTrap protocol (see below).

Column Activation and Endotoxin Removal

1. Regenerate the column with 6 column volumes regeneration buffer.
2. Wash the column with 6 column volumes equilibration buffer.
3. Apply sample onto the column and start collecting your fractions immediately. Applied sample elutes directly after the column void volume. Take care, that the max. flow rate is 0.2-1 ml/min and the max. pressure is 3 bar, 43 psi, 0.3 MPa, so that the resin does not compress. The slower the flow rate, the more efficient is the endotoxin removal. Afterwards let the sample drain completely from column.
4. In order to elute your entire sample, apply extra **equilibration buffer** (e.g. 1 column volume), let the column drain out and collect the flow through completely.

Column Regeneration

- Wash the column with 6 column volumes equilibration buffer (EB). Continue with step 1 of "Column Activation and Endotoxin Removal".

Column Storage

- If you want to store the column, apply 6 column volumes of storage buffer with 0.02% sodium azide or 2.5 ppm ProClin™. Then remove & close the column and store at 2-8°C.

EndoTrap® HD

EndoTrap® **HD** has been especially optimized for application in biomanufacturing processes. It can be used in early or late bio-manufacturing process steps. EndoTrap® **HD** is based on a hydrophilic, dimensionally stable affinity matrix with excellent pressure / flow characteristics. The LPS binding capacity of EndoTrap® **HD** is 5.000.000 EU/ml resin and each cleaning step theoretically yields a three log reduction of LPS. Also a Regulatory Support File can be provided.

For further information see our package inserts and the FAQs at www.lionex.de.

Technical Support and Further Product Information

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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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

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