

## 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/Mg<sup>2+</sup> 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.

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<br><i>we recommend &lt; 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 <b>freshly</b> with 1 mM Ca <sup>2+</sup> /Mg <sup>2+</sup> 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® HD works effectively in the pH range of 4-9 and NaCl concentrations of 50-600 mM.</p> <p>Buffers such as <b>HEPES, TRIS, MOPS, MES, and PIPES</b> are recommended. Citrate buffers and chelators of divalent cations (like <b>EDTA</b>) <b>have to be avoided</b>.</p> <p><b>Note:</b> Customer specific buffers must contain 0.1 - 1 mM <math>\text{Ca}^{2+}</math> or <math>\text{Mg}^{2+}</math>. When using <b>PBS buffer</b> always add 1 mM <math>\text{Ca}^{2+}</math> (e.g. <math>\text{CaCl}_2</math>) and 1 mM Citrate pH 7 freshly to the customer specific buffer especially. Otherwise phosphate and <math>\text{Ca}^{2+}</math> form an insoluble complex and will precipitate.</p> <p>For <b>DNA application</b> the following buffer composition is recommended:<br/>10 mM Tris-HCl, 1 mM <math>\text{CaCl}_2</math>, 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 <b>HEPES, PBS, TRIS, MOPS, MES, PIPES</b> and also <b>Citrate, Acetate, Glycine</b> and <b>Carbonate buffers</b> are recommended. EndoTrap® red can be used with chelators of divalent cations (like <b>EDTA</b>).</p> <p><b>Phosphate buffers with NaCl concentrations below 100 mM</b> are recommended for EndoTrap® red.</p> <p>With a “classical” PBS buffer (10 mM <math>\text{Na}_2\text{HPO}_4</math>, 1.8 mM <math>\text{KH}_2\text{PO}_4</math>, 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 <math>\text{Na}_2\text{HPO}_4</math>, 0.9 mM <math>\text{KH}_2\text{PO}_4</math>, 68.5 mM NaCl, 1.35 mM KCl, pH 7.4).</p> |

### Comparison of the specifications of 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"> <li>proteins</li> <li>peptides</li> <li>antibodies</li> <li>antigens</li> <li>plant extracts</li> <li>plasmid DNA / RNA</li> </ul> | <ul style="list-style-type: none"> <li>proteins</li> <li>peptides</li> <li>antibodies</li> </ul> |
| <u>Regeneration buffer (endotoxin concentration)</u><br>≤ 0.02 EU/ml  | Regeneration buffer “HD”<br>(based on “HEPES buffer”, pH 7.5)                                                                                                         | Regeneration buffer “red”<br>(based on “Phosphate buffer”, pH 7.4)                               |
| <u>Equilibration buffer (endotoxin concentration)</u><br>≤ 0.02 EU/ml | Equilibration buffer “HD”<br>[“HEPES buffer”, pH 7.5 enriched with 0.1 mM $\text{CaCl}_2$ ]                                                                           | Equilibration buffer “red”<br>[“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 $\text{Ca}^{2+}$ or $\text{Mg}^{2+}$ 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<br>< 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 <sup>6</sup> 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)<br>0.005% Tween20®<br>max. 0.005% NaDOC<br>max. 0.5 M GdnHCl<br>10% DMSO<br>20% Isopropanol<br>20% Methanol<br>20% Ethanol<br>10% Glycerol / Glycerin<br>0.5 M Urea (up to 2 M at pH 7 possible)<br>300 mM Imidazole                                                                                                                                                                                                                                                  | DTT not tested<br><br>max. 0.005% NaDOC<br><br>20% DMSO<br>20% Isopropanol<br>40% Methanol<br>20% Ethanol<br>20% Glycerol / Glycerin<br>1 M Urea<br>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"> <li>&gt; 10 mM NaOH</li> <li>SDS and other detergents</li> <li>Citrate</li> <li>EDTA, and other Calcium chelators (EGTA, HEDTA, NTA)</li> <li>Ammoniumsulphate</li> </ul>                                                                                                                                                                                                                                                                                           | <ul style="list-style-type: none"> <li>&gt; 250 mM NaCl</li> <li>SDS, Tween20 and other detergents</li> <li>GdnHCl</li> <li>Ammoniumsulphate</li> </ul>                                                                                                                                                                                       |
| <u>Tested LPS-types (bacterial strain)</u>                                                                                          | <ul style="list-style-type: none"> <li><i>Escherichia coli</i> K12, BL21,</li> <li>R1, R2, R3, R4,</li> <li><i>Salmonella enterica</i></li> <li><i>Citrobacter freundii</i></li> <li><i>Citrobacter amalonaticus</i></li> <li><i>Citrobacter koseri</i></li> <li><i>Pseudomonas aeruginosa</i></li> <li><i>Pseudomonas stutzeri</i></li> <li><i>Enterobacter aerogenes</i></li> <li><i>Enterobacter asburiae</i></li> <li><i>Enterobacter cloacae</i></li> <li><i>Aeromonas hydrophilia</i></li> </ul> | <ul style="list-style-type: none"> <li><i>Escherichia coli</i> K12</li> <li><i>Salmonella enterica</i></li> <li><i>Citrobacter freundii</i></li> <li><i>Pseudomonas aeruginosa</i></li> </ul> <p>Use EndoTrap red for:</p> <ul style="list-style-type: none"> <li><i>Klebsiella pneumoniae</i></li> <li><i>Serratia marcescens</i></li> </ul> |

\* 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                                    | <b>No incubation step</b> – real flow-through system                                                                                                                                                                                                           |
| Best protein recovery rate                   | <b>Minimized</b> sample loss, up to 99 % sample recovery                                                                                                                                                                                                       |
| LPS variability                              | Endotoxin removal from broad range of <b>bacteria strains</b>                                                                                                                                                                                                  |
| 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, <b>no Deoxycholate (DOC)*!</b>                                            |
| Temperature stability                        | Regular use in range between <b>4°C</b> and <b>room temperature (up to 35°C)</b>                                                                                                                                                                               |
| Buffers                                      | Buffers included in kit ( <b>EndoTrap HD 1/1, 5/1, EndoTrap red</b> )<br>buffer preparation protocol provided ( <b>EndoTrap HD</b> )                                                                                                                           |
| Kit included<br>(no extra cost for buffers!) | <b>EndoTrap HD 1/1, 5/1, EndoTrap red</b> : <i>ready to use</i> columns or resin, regeneration and equilibration buffers<br><b>EndoTrap HD</b> : 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   | <b>EndoTrap red</b> : 2.000.000 EU/ml resin<br>(each cleaning step theoretically yields a two log reduction of LPS)<br><b>EndoTrap HD</b> : 5.000.000 EU/ml resin<br>(each cleaning step theoretically yields a three log reduction of LPS) |
| Support matrix     | <b>EndoTrap red</b> : Highly cross-linked 4% sepharose, spherical beads<br><b>EndoTrap HD</b> : hydrophilic, cross-linked methacrylic polymer                                                                                               |
| Void volume        | 0.3 to 0.5 ml / ml resin                                                                                                                                                                                                                    |
| Mean particle size | 40 - 90 µm                                                                                                                                                                                                                                  |
| Max. flow rate     | <b>EndoTrap red</b> : 0.2 - 1 ml/min (gravity flow)<br><b>EndoTrap HD</b> : up to 100 ml/h/ml resin                                                                                                                                         |
| Max. pressure      | <b>EndoTrap red</b> : 3 bar, 0.3 MPa,<br><b>EndoTrap HD</b> : 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. <b>Do not freeze!</b>                                                                                                                              |
| Shelf life         | EndoTrap is stable until the stated expiry date if stored correctly at least 2 years from production date.                                                                                                                                  |

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- ♣ (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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### Products of the EndoTrap - family

| Product                                               | Contents                                                                                              | Cat. No. |
|-------------------------------------------------------|-------------------------------------------------------------------------------------------------------|----------|
| <b>EndoTrap HD 1/1</b>                                | 1 x 1-ml column, ready to use,<br>equilibration buffer, regeneration buffer, storage buffer           | LET009   |
| <b>EndoTrap HD 5/1</b>                                | 5 x 1-ml columns, ready to use, 5x equilibration buffer,<br>5x regeneration buffer, 5x storage buffer | LET0010  |
| <b>EndoTrap HD 5</b>                                  | 5 ml settled resin,<br>supplied as 50% slurry in regeneration buffer                                  | LET0023  |
| <b>EndoTrap HD 10</b>                                 | 10 ml settled resin,<br>supplied as 50% slurry in regeneration buffer                                 | LET0011  |
| <b>EndoTrap HD 50</b>                                 | 50 ml settled resin,<br>supplied as 50% slurry in regeneration buffer                                 | LET0012  |
| <b>EndoTrap HD 250</b>                                | 250 ml settled resin,<br>supplied as 50% slurry in regeneration buffer                                | LET0013  |
| <b>EndoTrap Leakage<br/>ELISA for EndoTrap<br/>HD</b> | 12 x 8 well stripes<br>1 vial POD-antibody<br>1 vial Standard, 20 ml ABTS substrate, ready to use     | LET0014  |
| <b>EndoTrap red 1/1</b>                               | 1 x 1-ml column, ready to use,<br>equilibration buffer, regeneration buffer                           | LET0001  |
| <b>EndoTrap red 5/1</b>                               | 5 x 1-ml columns, ready to use,<br>equilibration buffer, regeneration buffer                          | LET0002  |
| <b>EndoTrap red 5</b>                                 | 5 ml settled resin,<br>supplied as 50% slurry in regeneration buffer;                                 | LET0033  |
| <b>EndoTrap red 10</b>                                | 10 ml settled resin,<br>supplied as 50% slurry in regeneration buffer;                                | LET0003  |
| <b>EndoTrap red 50</b>                                | 50 ml settled resin,<br>supplied as 50% slurry in regeneration buffer;                                | LET0004  |

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### Customer feedback

Statements from EndoTrap® customers:

- ... “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.ilexlife.com](http://www.ilexlife.com)

### Trademarks

EndoTrap® is a registered international trademark of LIONEX GmbH.

EndoLISA® endotoxin detection is a registered international trademark of Hyglos GmbH - a bioMérieux company.

Tween20® is a registered trademark of ICI America, Inc.

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EndoTrap® patented technology is manufactured by LIONEX GmbH and is provided for research use only.

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