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

LIONEX Diagnostics and Therapeutics

Package Insert EndoTrap® HD Leakage ELISA

for the quantitative determination of EndoTrap® HD binding ligand

- **Cat. No. LET0014 - EndoTrap® HD Leakage ELISA:**
  - **LET0039 - EndoTrap® Leakage ELISA coated MTP**
  - **LET0040 - EndoTrap® Leakage ELISA POD-Antibody**
  - **LET0041 - ABTS Substrate, 20 mL, ready-to-use**
  - **LET0042 - EndoTrap® Leakage ELISA Standard**

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

Store the kits at +2 to 8 °C

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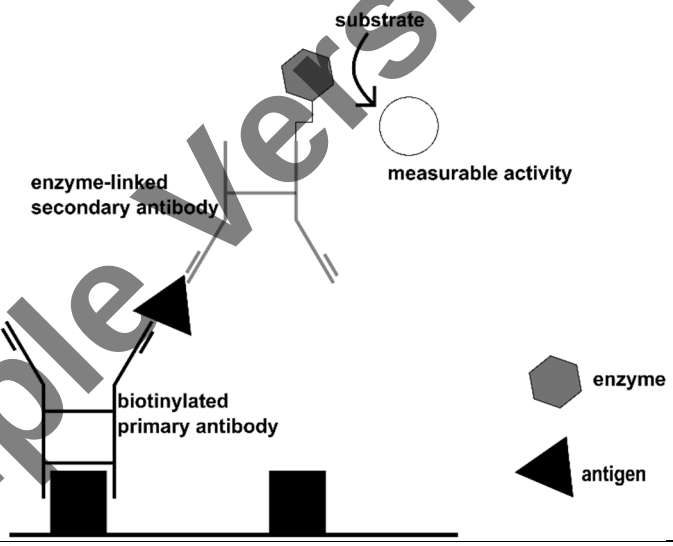
1. Introduction

EndoTrap® affinity chromatography is one principal method for cleaning biological solutions from endotoxin contaminations. 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 and testing on these contaminants is often required for regulatory purposes. Depending on the intended use of the preparation and the step in the purification process (early or late), where EndoTrap® is used, a quantitative analysis of residual EndoTrap® ligand might be required. The EndoTrap® HD Leakage ELISA was 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.

2. Principle

Test principle	The EndoTrap® HD Leakage ELISA is a two-step sandwich enzyme-linked immunosorbent assay for the quantitative determination of EndoTrap® ligand in biological aqueous solutions. The biotinylated capture antibody has been pre-coated to the streptavidin surface of the microtiter plate. The detection antibody is directly conjugated to the marker enzyme (peroxidase). ABTS is used as chromogenic substrate.
Antigen = EndoTrap® ligand Enzyme = POD Substrate = ABTS	

- ABTS = 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)

3. Package size

EndoTrap® HD Leakage ELISA	
Pack size:	96 determinations (12 x 8 well strips & frame)
Kit components:	1. **EndoTrap® Leakage ELISA coated MTP**: Streptavidin microtiter plate, pre-coated with EndoTrap® ligand-specific monoclonal antibody 2. **EndoTrap® Leakage ELISA POD-Antibody**: Peroxidase-conjugated monoclonal antibody specific for EndoTrap® ligand (lyophilized) 3. **ABTS Substrate**, 20 mL (ready to use) 4. **EndoTrap® Leakage ELISA Standard**: Standard protein (lyophilized) 5. **Package insert** with working instructions

4. Additional required solutions and equipment

Washing buffer
Conjugate buffer
Sample dilution buffer
Pipettes
Adhesive cover foils
MTP shaker
MTP reader
MTP washer (optional)
ELISA calculation software (recommended)

5. Specifications

Intended use	Quantification of EndoTrap® HD ligand leakage in biological aqueous solutions.
Specifity	Two specific monoclonal antibodies to EndoTrap® ligand (EndoTrap® HD or EndoTrap® blue) 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 31.25 pg/mL
Limit of Quantification (LOQ)	31.25 pg/mL EndoTrap® ligand
Assay time	The assay time is approximately three hours.
Standard protein	EndoTrap® blue ligand
Shipment condition	Ambient temperature
Storage condition	At 2-8°C. **Do not freeze!**
Shelf live	Unused material is stable until the stated expiry date when stored correctly (at 2-8°C).

6. Preparation of buffer

Content	Reconstitution	Stability
Sample dilution buffer	20 mM HEPES, 150 mM NaCl, 0,1 mM $CaCl_2$, 1% BSA, pH 7.5	3 months at 2-8°C, *sterile filtrated*
Conjugate buffer	20 mM HEPES, 150 mM NaCl, 1% BSA, pH 7.5	3 months at 2-8°C, *sterile filtrated*
Wash buffer	20 mM HEPES, 150 mM NaCl, 0,05% Tween20, pH 7.5	3 months at 2-8°C, *sterile filtrated*

7. Preparation of working solutions

POD-Antibody	Stock solution: Reconstitute the lyophilized POD- Antibody (0.75 U) in 1 mL double dist. water, let sit for 10 min at RT then mix thoroughly. **Do not vortex.**	1 month at 2-8°C 3 months at -20°C
	Working solution: The stock solution has to be diluted 1:15 in conjugate buffer	Prepare freshly
Standard dilution series	Stock solution: Reconstitute the lyophilized Standard (20 ng) in 1 mL double dist. water for 10 min at RT and mix thoroughly (20 ng/mL). **Do not vortex.**	1 month at 2-8°C 3 months at -20°C
	Dilution series: The stock solution (20 ng/mL) has to be diluted 1:10 with sample dilution buffer (see section Sample Preparation) to a starting final concentration of 2000 pg/mL. Then prepare a dilution series in 1:2 dilution steps: 2000 / 1000 / 500 / 250 / 125 / 62,5 / 31,25 / 0 pg/mL	Prepare freshly

8. Preparation of sample

General guidelines	- If EndoTrap® HD resin has been equilibrated and regenerated accordingly, the concentration of leached ligand in fractions or pools should be in the range of 300 pg/mL to10.000 pg/mL. - When applying concentrated sample solutions (e.g. > 5 mg/mL) the concentration of leached ligand could be higher than 10.000 pg/mL in the very first fraction. - To reach very low levels of leakage, **the first column volume of the preparation may be discarded**. - To avoid interference of the sample-specific buffer system with the ELISA determination, it is recommended to dilute samples 1:5 in sample dilution buffer. - For determinations of **undiluted samples**, it is recommended to set up the standard curve in the customer-specific buffer system (extreme pH, high salt, detergents or reducing agents may interfere with the determination). - Mix diluted samples thoroughly before pipetting into the wells.

9. General Remarks

Recommendations	- Perform a **standard curve** with each test series. - For standard curve as well as samples a duplicate determination is recommended. - Use **positive controls** (sample spiked with LPS) to evaluate the influence of your sample on the ELISA. - Use **negative controls** (buffer) to evaluate the influence of your buffer on the ELISA. - Use only calibrated pipettes. - Make sure that all reagents and buffers used in the ELISA have **room temperature**. - The ABTS substrate is very sensitive to contaminations. Do not pipette directly from the bottle. We recommend transferring the required amount into a separate container. - We recommend using a **non-linear curve fitting program** for analysis.

10. ELISA Protocol

Step	Action	Volume / Well	Incubation time
1	**Wash plate three times** with washing buffer to hydrate the plate.	3 x 250 µL	3 x 1 min.
2	**Remove the washing fluid** by aspiration or thorough tapping.		
3	**Pipet standards and samples** accurately into the wells and cover the stripes tightly with an adhesive cover foil. Incubate at room temperature under constant shaking at 600 rpm.	100 µL	60 min
4	**Remove the solution** by aspiration. Alternatively, the stripes may be inverted and tapped gently on a paper towel. Wash three times with washing buffer.	3 x 250 µL	3 x 1 min.
5	**Remove the washing fluid** by aspiration or tapping.		
6	**Pipet POD-Antibody** working solution accurately into each well, cover the stripes tightly with adhesive cover foils and incubate at room temperature under constant shaking at 600 rpm.	100 µL	60 min
7	**Remove the solution** by aspiration. Alternatively, the stripes may be inverted and tapped gently on a paper towel. Wash three times with washing buffer.	3 x 250 µL	3 x 1 min.
8	**Remove the washing fluid** by aspiration or thorough tapping.		
9	**Pipet ABTS substrate solution** accurately into each well and incubate under constant shaking at 600 rpm.	100 µL	10-30 min
10	**Photometric measurement**: Measure at 405 nm (reference wavelength 620 nm) in intervals of 5 min (readings can be stopped when the highest standard concentration reaches OD = 2.0)		
11	**Analysis**: The measured values should be within the range of the standard curve (between 31.25 and 2000 pg). For data calculation we recommend using a non-linear curve fitting program.		

11. Standard curve

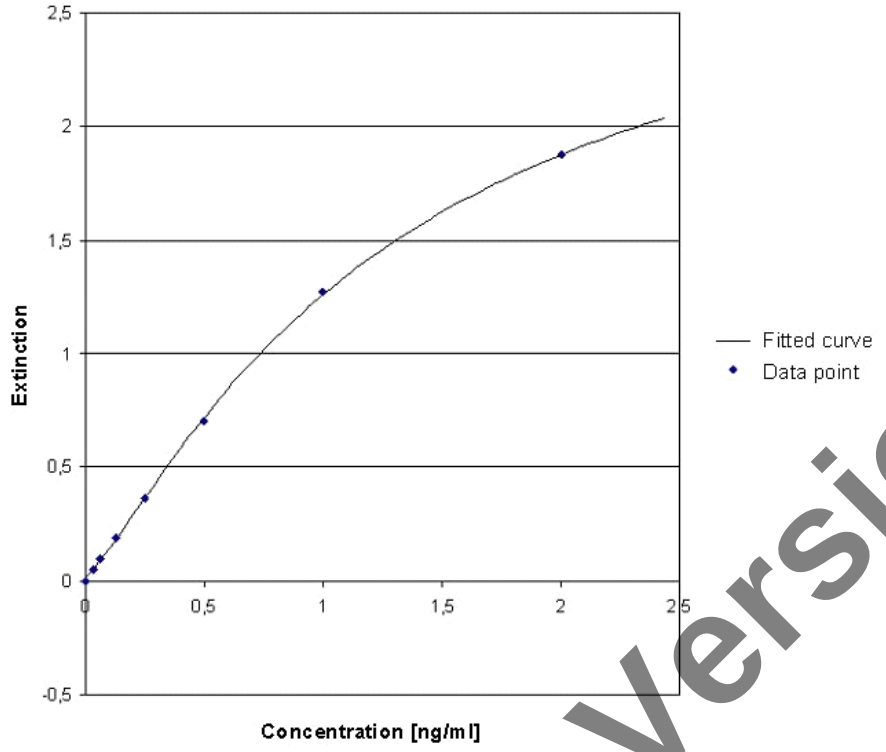


Figure 1: Typical standard curve: The measured values must be within the standard curve (between 31.25 and 2000 pg/mL) to be valid. Otherwise the assay should be repeated with another sample dilution.

12. Trouble shooting

Problem	Possible Cause	Recommendation
Unexpected colour development	Inadequate incubation time and temperature	Ensure that incubation-intervals are correct and that all reagents achieve RT before using in the test
	Uncontrolled water ingredients influence the test negatively	Always use double distilled water for reconstitution and preparing the working solutions; take care that the water is not microbially contaminated
	Substrate or vial used to aliquot substrate is contaminated with oxidative active substances	Do not pipet directly from the substrate bottle! Check the vial for contamination!
	Inadequate concentration of conjugate in the working solution	Adjust to the correct concentration of the detection antibody in the working solution
Weak or no signal	Sodium azide, ß-mercaptoethanol, and DTT interfere with the peroxidase-activity	Only use samples and solutions without sodium azide, ß- mercaptoethanol or DTT
Drift	Unequal distribution of temperature in the wells	Ensure that all reagents achieve RT before use, and keep the recommended incubation times and temperatures
	Evaporation of fluids	Check the adequate fixation of the adhesive cover foils during the incubation steps
Poor precision	Non-homogeneous sample after freezing	Mix sample well before pipetting
	Turbidity, particles or high lipid content of the sample	Centrifuge sample to pellet particles. Transfer supernatant. Mix sample well before pipetting.
	Carry over between samples / standards	Change pipette tips between each pipetting steps.
	Unequal volumes added to the wells	Check pipette function, and recalibrate if necessary
	Inadequate aspiration of fluids	No fluid should remain in the wells after aspiration
	Washing was incomplete	Ensure that the automatic washer is working properly
	Unequal mixing of reagents during incubation	Use a plate shaker to ensure adequate mixing
Questionable readings	Non-suitable filters in the MTP reader have been used	Check the filters in your MTP reader for the correct wavelength

13. 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

- **EndoTrap® HD** Endotoxin removal system for High-Definition sample purification

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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