

Hycult biotechnology

## EndoClear Profos EndoTrap<sup>®</sup> and HBT Limulus Amoebocyte Lysate Chromogenic Endpoint Assay (LAL Assay)

**Catalog No.** HIT305 (EndoTrap<sup>®</sup> blue 1/1 and HBT mini-LAL Assay)

**Quantity :** 1 x 1ml column

**Description** Bacterial endotoxin, lipopolysaccharide (LPS), is a fever-producing by-product of gram-negative bacteria commonly known as pyrogen. LPS consists of a polysaccharide, a core oligosaccharide and lipid A, which is responsible for the toxic effects. In human, lipopolysaccharide binding protein (LBP) plays a central role in response to LPS in the activation pathway and in the neutralization of LPS. Removal of endotoxin is one of the most difficult downstream processes during protein purification. EndoTrap<sup>®</sup> blue, as part of the EndoClear kit, is developed to meet the most challenging requirements of endotoxin removal: the highest endotoxin removal capacity combined with excellent recovery rates. EndoTrap<sup>®</sup> blue is based on affinity chromatography for column or batch mode. The working range of EndoTrap<sup>®</sup> blue is pH 4-9 and it works with an ionic strength up to 600 mM NaCl. Proteins, peptides, antibodies and plasmid DNA can be applied onto the EndoTrap<sup>®</sup> blue column. Customer specific buffers can be used when enriched freshly with Ca<sup>2+</sup>. Besides the EndoTrap<sup>®</sup> blue, EndoTrap<sup>®</sup> red is also available. EndoTrap<sup>®</sup> red has a pH range of 6-9 and is capable to work with an ionic strength up to 250 mM (<100 mM is recommended). LPS from *Klebsiella pneumoniae* and *Serratia marcescens* can only be removed with EndoTrap<sup>®</sup> red.

The detection of endotoxin is based on studies of Frederick Bang. He observed that bacteria caused intravascular coagulation in the American horseshoe crab, *Limulus polyphemus*. In collaboration, Levin and Bang found that the agent responsible for the clotting phenomena resided in the crab's amoebocytes, or circulating blood cells, and that pyrogen triggers the turbidity and gel-forming reaction enzymatically.

The Mini-LAL assay, the second part of the EndoClear kit, makes use of an activated enzyme. In the presence of a colorless substrate, the enzymatic reaction will cause a yellow color to develop upon cleavage of the chromophore, p-nitroaniline (pNA). The reaction is stopped by the addition of acetic acid and the absorbance at 405 nm is measured with a spectrophotometer. The endotoxin concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from a standard curve.

**Application** The EndoClear kit provides the opportunity to remove and detect endotoxin with just one kit. EndoClear consists of the Profos EndoTrap<sup>®</sup> blue assay and the Hbt Mini-LAL assay. The Profos EndoTrap<sup>®</sup> blue assay is intended for the removal of endotoxin with removal rates of >95% per cycle. The binding capacity of EndoTrap<sup>®</sup> blue is 2,000,000 EU/ml (1EU = 100 pg LPS) The Hbt Mini-LAL assay is intended for the quantitative measurement of endotoxin in culture medium, buffers, and other solutions. The kit has a minimum detection limit of 0.01 EU/ml and a measurable concentration range of 0.01 to 10 EU/ml.

**Features** Profos EndoTrap<sup>®</sup> blue assay:

- Binding capacity of 2,000,000 EU/ml.
- Removal rates of >95% per cycle.
- Final LPS concentration 0.005 – 0.1 EU/ml.
- Column or batch mode.
- Operating at pH 4.0-9.0.
- Suitable for plasmid DNA, proteins, peptides and antibodies.
- Suitable for an ionic strength up to 600 mM NaCl.
- Customer specific buffers can be used when enriched freshly with Ca<sup>2+</sup>.



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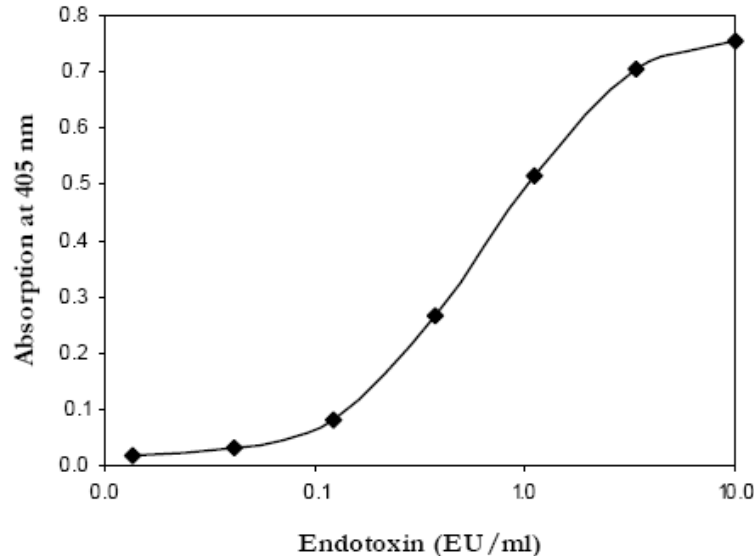
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Hbt Mini-LAL assay:

- Minimum concentration which can be measured is 0.01 EU/ml.
- Measurable concentration range of 0.01-10 EU/ml.
- Working volume of 50 µl/well.
- 72 determinations
- Operating at pH 6.5-8.0.

### Typical Standard Curve

### Mini-LAL Chromogenic Endpoint Assay



### Principle

Profos EndoTrap® blue assay:

- The Profos EndoTrap® blue Assay is based on affinity chromatography with a working time of ~30 minutes.
- Sample is applied to the column with equilibration buffer.
- Fractions are collected.
- Removal rate is >95% per cycle.
- Samples are measured with Hbt LAL Assay.

Hbt Mini-LAL assay:

- The LAL assay is an enzyme-based assay with a working time of ~45 minutes.
- Samples and standards are incubated with LAL reagent.
- The enzymatic reaction, triggered by endotoxin, will cause a yellow color to develop upon cleavage of the chromophore, p-nitroaniline.
- The enzymatic reaction is stopped by the addition of acetic acid.
- The absorbance at 405 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the *E. coli* standards (log).
- The endotoxin concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

### Storage and stability

Products should be stored at 2 – 8 °C. Under recommended storage conditions, products are stable for at least six months.

The Profos EndoTrap® blue columns should be stored in regeneration buffer with 0.02% sodium azide. After reconstitution the Hbt Mini-LAL assay reagents (*E. coli* standard and LAL reagent) should be stored in aliquots at -20 °C.



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- Recovery** Recovery for the Hbt Mini-LAL assay is evaluated by interference (inhibition/enhancement) testing through spiking a sample or diluted sample with a known concentration of endotoxin and testing for spike recovery in duplicate following the assay procedure. In general, serum samples contain interfering factors. Therefore, we recommend to perform recovery experiments to confirm the reliability of endotoxin detection in serum.  
For interference testing select a point at or near the middle of the standard curve. The calculated mean amount of endotoxin in the spiked sample, when referenced to the standard curve, must be within 50-200% to be considered free of inhibition or enhancement. Failure to recover the spike within 50-200% indicates sample interference. Further dilute the sample in endotoxin free water until the spike is recovered consistently by the assay.
- Precautions** For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and Federal rules in the use of this product. Hbt is not responsible for any patent infringements that might result with the use of or derivation of this product.
- References**
1. Savill, J et al; Phagocyte recognition of cells undergoing apoptosis. *Immunol Today* 1993, 14:131
  2. Reutelingsperger, C et al; Annexin V, the regulator of phosphatidylserine-catalyzed inflammation and coagulation during apoptosis. *Cell Mol Life Sci* 1997, 53:527
  3. Defrancesco, L et al; Dead Again: Adventures in Apoptosis. *The Scientist* 1999, 13: 17
- Also available**
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|--------|--|
| HIT306 | EndoClear EndoTrap red 1/1 and Mini-LAL Assay  |
| HIT307 | EndoClear EndoTrap blue 5/1 and Mini-LAL Assay |
| HIT308 | EndoClear EndoTrap red 5/1 and Mini-LAL Assay  |
| HIT309 | EndoClear EndoTrap blue 10 and Mini-LAL Assay  |
| HIT310 | EndoClear EndoTrap red 10 and Mini-LAL Assay   |
| HIT302 | LAL Chromogenic Endpoint Assay                 |

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