Endotoxin Removal and Quantitation

*Endotoxin contamination is a common problem with recombinant proteins purified from gram-negative bacteria such as E. coli. Even low levels of endotoxins can be toxic to cells or organisms and must be removed before biological samples can be introduced. Interchim selected efficient supports to selectively bind and removes ≥99% of endotoxins from protein, peptide and antibody samples.*

*ToxinEraser™ Endotoxin Removal Kit*
A complete kit to remove efficiently endotoxins (LipoPolySaccharide) from biological samples.

*Detoxi-Gel Endotoxin Removal Gel and columns*
Use affinity of Polymixin B grafted on agarose beads.

*High Capacity Endotoxin Removal Resin and columns*
Use affinity of modified ε-poly-L-lysine [poly(ε-lysine)] grafted on porous cellulose beads.

*LAL Chromogenic Endotoxin Quantitation Kit*
*ToxinSensor™ Gel Clot Endotoxin Assay Kit*
*LAL Chromogenic Endotoxin Quantitation Kit*

Endotoxins are lipid portions of LipoPolySaccharides (LPSs) that are part of the outer membrane of the cell wall of gram-negative bacteria, liberated when the bacteria die and the cell wall breaks apart. They are not *exotoxins*, proteins produced inside pathogenic bacteria (mostly gram-positive) as part of their growth and metabolism, and secreted or released in the surrounding medium following lysis.

Endotoxins are often **mistakenly considered to be absent or not significant**, below evident toxic concentration (e.g. cells affected at 100ng/ml): they in fact affect cells at much lower levels, for example inhibiting differentiation of hESC to mesoderm (5pg/ml), or altering hematopoietic precursor cells in culture (2pg/ml) and even at 0.01pg/ml altering physical behaviour of endothelial cells (4).
ToxinEraser™ Endotoxin Removal Kit

**Description**

ToxinEraser is an endotoxin removal resin of high efficiency. It is based on the affinity matrix of modified polymyxin B (PMB), which allows highly efficient endotoxin removal. The final endotoxin level can be decreased to as low as 0.1 EU/ml * with repeat use of ToxinEraser™ endotoxin removal resin. * final removal efficiency may vary depending on the sample type/source.

**Key Features**

- High stability and high removal efficiency
- High binding capacity: > 2 000 000 EU/ml (CV)
- Fast flow without any constant speed pump
- Reusable up to five times if properly regenerated
- Ready-to-use reagents and materials, such as equilibration buffer, collection tubes, etc.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Product Name</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>QZ9690-L00402</td>
<td>ToxinEraser™ Endotoxin Removal Resin</td>
<td>1 ml</td>
</tr>
<tr>
<td>RA0830-M01053</td>
<td>ToxinEraser™ Regeneration Buffer</td>
<td>125 ml</td>
</tr>
<tr>
<td>RA0840-M01054</td>
<td>ToxinEraser™ Equilibration Buffer</td>
<td>125 ml</td>
</tr>
<tr>
<td>VGF490-M01063</td>
<td>ToxinSensor™ Endotoxin-free Pipette Tips (1 ml, Blue)</td>
<td>1 PK of 6 tips</td>
</tr>
</tbody>
</table>

**FEATURES:**

- 1.5 ml resin pre-packed column | Binding Capacity up to 2 000 000 EU LPS / ml resin
- [4% cross-linked agarose, spherical beads, with mean particle size 90 μm, coupled to modified PMB (Polymyxin B) ligand coupled to ]
- Stable at pH 5 to 10
- Contains Resin filled columns, all needed buffers, collection Tubes and Tips, Flow-Speed Control
- Storage 2 °C to 8 °C (Do not freeze) - Shelf Life 18 months

**OPERATING:**

- Equilibrate the column in provided Buffer (Phosphate Buffer pH 8.0)
- Inject sample (Protein including peptides and antibody; polysaccharide etc) - Applicable Ionic Strength: 0.1 to 0.5 M NaCl - Compatible with: 20% DMSO; 20% ethanol; 20% glycerol; 1 M urea; 300 mM imidazole; 0.05% Tween 20, 10 mM DTT.

**References**


W Gong, et al. Surface protein Adr2 of <i>Rickettsia rickettsii</i> induced protective immunity against Rocky Mountain spotted fever in C3H/HeN mice. Vaccine. 2014Feb.;

Detoxi-Gel Endotoxin Removal Gel

Eliminate worries about endotoxins interfering with your test results.

Detoxi-Gel Endotoxin Removing tools provide a quick method for removal of endotoxins (lipopolysaccharides or LPS) from biological samples. Resin uses immobilized polymixin B to remove pyrogens by binding their lipid A domains.

Benefits:

- **Efficient removal of Endotoxin, LPS, pyrogens**
  1 ml of gel removes >99.95 EU from a 10,000 EU challenge of LPS (greater than 99% efficiency)
  1 ml of gel binds 4000-6000 E.U. of the lipopolysaccharide from E. Coli strain 055:BS

- **Quickly reduces endotoxin levels with >85% Recovery of many samples.**
  Effective in protein solutions, cell culture media, solutions containing pharmacological components and aqueous buffers
  Protein recovery dependent upon protein type and concentration; some empirical testing required

- **Stable and reusable**
  The resin can be regenerated by stripping off the endotoxins with a 1% deoxycholate solution in pyrogen-poor water; no loss in binding capacity even after 10 regenerations
  Available as a slurry to pack a custom column or in convenient pre-packed, single-use spin columns optimized for different sample volumes.

**Detoxi-Gel Endotoxin Removing Gel**

Detoxi-Gel Endotoxin Removing Gel 408410, 10mL 408412, 1L
Polymixin B on crosslinked 6% beaded agarose slurried in 25% ethyl alcohol
Sufficient For: Binding approx. 10,000 endotoxin units of LPS per mL of resin per use

**Detoxi-Gel Endotoxin Removing Columns**

L76971, 1 mL
5mL-capacity columns containing 1mL of resin
Sufficient for binding approx. 10,000 endotoxin units of LPS per column per use

**High Capacity Endotoxin Removal Gel**

Endotoxin levels in biological samples are reduced by ≥99% in as fast as 1 hour using our spin column format, and protein recovery is ≥85%. It uses modified ε-poly-L-lysine [poly(ε-lysine)] affinity grafted onto porous cellulose.

High Capacity Endotoxin Removal Resin is available as a slurry to pack a custom column or in convenient pre-packed, single-use spin columns optimized for different sample volumes.

- **High capacity** – bind up to 2,000,000 EU/mL to eliminate >99% of endotoxins
- **Durable** – Stable resin, reusable up to 10 times
- **Selective** – recover ≥85% of your protein sample
- **High performance** – complies with FDA guidelines by reducing final EU concentration to <5 EU/mL
- **Fast** – our new spin column format enables endotoxin depletion within 1 hour
- **Clean** – single-use spin columns avoid cross contamination of samples
- **Optimized** – spin columns are optimized for different sample volumes
- **Economical** – large-volume discounts available

**High Capacity Endotoxin Removal Resin**

KV5611, 10mL KV5612, 100mL
Proprietary ligand on beaded cellulose slurried in 20% ethanol
Sufficient for removing >99.9% of 10,000 endotoxin units (per mL of resin) from 5mL test samples

**High Capacity Endotoxin Removal Spin Columns, 0.25mL**

IUE780, 5 columns
5mL-capacity centrifuge columns containing 0.25mL resin
Sufficient for removing >99.9% of the endotoxin present in typical 0.5 to 1mL samples

**High Capacity Endotoxin Removal Spin Columns, 0.5mL**

IUE781, 5 columns IUE782, 25 columns
8mL-capacity centrifuge columns containing 0.5mL resin
Sufficient for removing >99.9% of the endotoxin present in typical 1 to 4mL samples

**High Capacity Endotoxin Removal Spin Columns, 1mL**

IUE5621, 5 columns IU5622, 25 columns
22mL-capacity centrifuge columns containing 1mL resin
Sufficient for removing >99.9% of the endotoxin present in typical 2 to 10mL samples
LAL Chromogenic Endotoxin Quantitation Kit
ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit is designed to be a quantitative In Vitro end-point endotoxin test for human and animal parenteral drugs, biological products, and medical devices. This method utilizes a modified Limulus Amebocyte Lysate and a synthetic color producing substrate to detect endotoxin chromogenically in a broad range of 0.005 - 1 EU/ml. In addition, any sample with color (e.g. cell bacterial culture medium, serum or blood etc.) cannot be assayed by this kit.

Key Features
- **Highly Sensitive and linear**: Detect 0.005 - 1 EU/ml endotoxin concentrations
- **Fast**: The incubation period can be shortened to 14 minutes
- **Reliable**: Color-stabilizer ensures accurate results
- **Ready-to-use**: Kit includes endotoxin-free tips and tubes, LAL reagent water and incubation rack
- **Broad application**: Quantitative in vitro end-point endotoxin test

References


ToxinSensor™ Gel Clot Endotoxin Assay Kit

Description
ToxinSensor™ Gel Clot Endotoxin Assay Kit is intended as an In Vitro end-product endotoxin test for human and animal parenteral drugs, biological products, and medical devices. The Limulus Amebocyte Lysate (LAL) test is a qualitative test for Gram-negative bacterial endotoxin. Limulus Amebocyte Lysate as supplied is to be reconstituted with LAL Reagent Water and then mixed in equal parts with the solution being tested. After incubation, and in the presence of endotoxin, gelation occurs; in the absence of endotoxin, gelation does not occur.

The procedures described conform with those described in the FDA Guideline. Similar performance requirements for gel clot assays have been published and are updated regularly in the United States Pharmacopeia.

Note: For specimen preparation, the specimen should be certified free of Beta Glucans contaminant which may come from yeast and cellulosic materials, such as blood products.

Key Features
- **Good reproducibility**
- **Ready-to-use reagents and materials, such as tips, Endotoxin-free tubes, etc**

References


Tech Sheet
LAL Chromogenic Endotoxin Quantitation Kit

Measure endotoxin levels in your protein sample quickly and accurately.

The LAL Chromogenic Endotoxin Quantitation Kit measures the amount of endotoxin in a protein, peptide or antibody sample using the Limulus Amebocyte Lysate (LAL) assay.

The endotoxin concentration in a sample is measured using the LAL Chromogenic Endotoxin Quantitation Kit via a chromogenic signal generated in the presence of endotoxins. Samples can be measured on a microplate absorbance reader at 405nm. A standard curve is created using the E. coli endotoxin standard included with each kit to calculate endotoxin levels as low as 0.1 EU/mL, where one endotoxin unit/mL (EU/mL) equals approximately 0.1ng endotoxin/mL of solution. Protein and antibody samples can be assayed in about 30 minutes. Determining endotoxin levels is important to assess the efficiency of endotoxin removal methods and prevent endotoxic shock, inflammation and/or sepsis in tissue culture cells and animals injected with endotoxin contaminated proteins.

Highlights:
- **Sensitive** – detect as little as 0.1 EU/mL (approx. 0.01ng endotoxin per mL)
- **Fast** – perform this assay in 30 minutes using a 96-well microplate
- **Economical** – assay requires only 10µL of a protein sample
- **Accurate** – E. coli O111:B4 standard in each kit enables accurate endotoxin quantitation
- **Versatile** – 405nm absorbance reading is compatible with common ELISA plate readers

- **Applications:**
  Quantitation of endotoxin levels in a protein, peptide or antibody solution

- **Ordering Information**
  **LAL Chromogenic Endotoxin Quantitation Kit**  
  IUE790-88282  50-test kit
  Multi-component kit, sufficient For: 30 microplate-well assays with 50µL samples containing 0.1 to 1 endotoxin units/mL
  Kit Contents:  
  Limulus Amebocyte Lysate (LAL), 2 vials  
  E. coli O111:B4 Endotoxin, 1 vial  
  Chromogenic Substrate, 1 vial  
  Endotoxin-free water, 1 vial

- **Product Details:**

  The LAL method for measuring endotoxin is based on the interaction of endotoxins with the proenzyme Factor C found in circulating amebocytes of the horseshoe crab Limulus polyphemus. The proteolytic activity of this proenzyme is activated in the presence of lipopolysaccharides (endotoxins) derived from the outer cell membrane of gram-negative bacteria such as E. coli. The Chromogenic Limulus Amebocyte Lysate assay measures endotoxin levels by measuring the activity of this protease in the presence of a synthetic peptide substrate that releases p-nitroaainiline (pNA) after proteolysis, producing a yellow color that can be measured by reading the absorbance at 405nm.

  To accurately measure endotoxin levels in a sample, the LAL assay uses an endotoxin standard of known concentration that is derived from E. coli strain O111:B4. This standard is provided with each kit and is used to create a standard curve. The endotoxin concentration is determined by extrapolating the absorbance of an unknown sample against this standard curve, similar to ELISA or total protein quantitation assays.

Endotoxin Quantitation Reaction Scheme

LAL Chromogenic Endotoxin Quantitation Kit reaction scheme. A small volume of the sample (10µL) is combined with the Limulus Amebocyte Lysate, and endotoxins in the sample activate the proteolytic activity of Factor C. When the chromogenic substrate is added, the activated protease catalyzes the cleavage of p-nitroaainiline (pNA), resulting in yellow color that can be quantitated by measuring the absorbance at 405nm (A405) and extrapolating against a standard curve.
More about Endotoxins

Endotoxin test is the most critical quality control test required by the FDA for all drugs in their final stages of formulation. Endotoxins are invariably associated with every gram-negative bacteria, so they cause severe reactions in humans and animals and retain high toxic activity even present at low concentration. In addition, endotoxins are suspected to play an important role in the occurrence and development of many different diseases.

Endotoxin FAQ

What is Endotoxin?

Endotoxin is a complex lipopolysaccharide (LPS), found on the outer membrane of most pathogenic Gram-negative bacteria. Although boiling for 30 minutes does not destabilize endotoxin, it can be degraded by certain powerful oxidizing agents, such as superoxide, peroxide and hypochlorite.

How do I convert EU/ml to ng/ml of endotoxin?

Endotoxin Units (EU), rather than units of weight, is a measure of the activity of endotoxin. It was developed by the FDA for comparison testing because the potency of endotoxin depends on a variety of factors: polysaccharide chain length, aggregation, solubility in biological fluids, bacterial source, associated substances, etc. Expressing endotoxin concentrations in EU addresses the issue of different potencies of different endotoxins.

Please use the following example to convert your endotoxin level: 10 EU/mL = 1.0 ng/mL.

Which endotoxin detection method should I choose?

Three things to consider before choosing an endotoxin detection method: the type of sample to be tested, its interference characteristics, and the endotoxin limit that you want to detect. The Gel-clot method is the simplest and most widely used method for endotoxin detection, and its sensitivity is up to 0.03 EU/ml. The Chromogenic method defines its sensitivity to be the lowest endotoxin concentration in the standard curve and requires a spectrophotometric instrumentation to read the results.

What is the maximum acceptable endotoxin level?

One EU is approximately equivalent to 100 pg of E. coli lipopolysaccharide—the amount present in approximately 105 bacteria. Humans can develop symptoms when exposed to as little as 5 EU/kg body weight. These symptoms include, but are not limited to, fever, low blood pressure, increased heart rate, and low urine output. Even small doses of endotoxin in the blood stream are often fatal.

The FDA has set the following maximum permissible endotoxin levels for drugs distributed in the United States:

Drug (injectable, intrathecal) - 0.2 EU/kg body weight  
Drug (injectable, non-intrathecal) - 5 EU/kg body weight  
Sterile water - 0.25-0.5 EU/ml (depends on intended use)

Does any substance interfere with the binding of endotoxin to endotoxin Removal Resin?

Yes. Other detergents and high levels of chaotropes (urea and guanidine) can reduce the affinity of Polymixin B for LPS. Proteins such as BSA can bind tightly to endotoxin, reducing its ability to interact with and bind to the endotoxin Removal Resin. This reduction in binding capacity can sometimes be overcome by increasing the volume of immobilized polymixin B. However, some proteins bind tightly to endotoxin without inhibiting its binding to ligands. In this case, the protein will remain bound to the resin with the endotoxin, possibly causing a loss of the protein of interest.

Related products/documents

Search at www.interchim.com  
See Products Highlights Overview
Information inquire

Reply by Fax: +33 (0) 4 70 03 82 60 or email at interbiotech@interchim.com

☐ I would like to receive further information on: __________________________________________________________

____________________________________________________________________________________________________

Title: _______ First name: ___________ Surname: ___________ Position: _________________________________

Company/Institute: _______________________________ Service, Lab: _________________________________

Adress: ________________________________________________

Postcode: _______ Town: ________________________________________________

Tel___________________ Fax___________________ Email: __________________________________________