Endotoxin Removal: New Solution for Protein Purification

EndoTrap®: Effective, Quick, Superior Recovery Rate

By Stephanie Steck, Ph.D.

Characteristics of Endotoxin

Lipopolysaccharides (LPS), or endotoxins, are biologically active structural components of the outer cell membrane of all Gram-negative bacteria. Endotoxins are invariably associated with Gram-negative bacteria whether the organisms are pathogens or not. Although the term “endotoxin” is occasionally used to refer to any cell-associated bacterial toxin, it is usually reserved to refer to the endotoxin/LPS complex associated with the outer membrane of Gram-negative bacteria.

Endotoxins are very stable molecules in comparison to proteins, with their biologically active part surviving extremes of temperature and pH. Baking of contaminated glassware is the recommended procedure to destroy endotoxin/LPS. However, it is not always practical to bake proteins and bioreagents since these will be destroyed as well.

Endotoxins are secreted by bacteria or released by lysis of the bacterial cell. They show high specificity (e.g. neurotoxins, enterotoxins, and cytokines) and toxicity in very low concentrations.

LAL Research - a Brief History of Endotoxin Detection

The discovery of perhaps the horseshoe crab’s most important role in human medicine was made by Frederick Bang in the early 1950s. Bang observed that the blood cells (called amebocytes) of the horseshoe crab contain a clotting agent that gels in the presence of endotoxins produced by Gram-negative bacteria. Bang’s studies on horseshoe crabs showed that the amebocyte cells in horseshoe crab blood belong to a primitive immune system. When a crab is injured, the amebocytes swarm to that area and coagulate, forming a viscous gel surrounding the invading bacteria. Unable to escape, the bacteria are

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soon destroyed by defense molecules such as antimicrobial proteins and polypeptides. This blood-clotting mechanism inhibits infection within the horseshoe crab. Bang recognized this clotting agent could be used as a fast and definitive way to test pharmaceutical drugs for the presence of Gram-negative bacteria. Up until then, drugs were tested by injecting rabbits with the drug and then waiting 48 hours to see if they produced a fever. Within a few years of his primary discovery, Dr. Bang and Dr. Levin had developed Limulus Amebocyte Lysate, or LAL, a novel method to test for Gram-negative bacteria. It was so effective that the U.S. Food and Drug Administration (FDA) accepted it as a standard test for endotoxins in 1983. Since then, LAL has gained widespread use, displacing rabbit tests for clinical and biomedical applications.

To prepare the lysate required for the LAL test, horseshoe crabs are caught from the sea bottom, and a small amount of their blood is drawn. The animals are then returned unharmed to the sea. The crabs’ blood cells (amebocytes) are isolated and lysed to release the cellular proteins.2,3

**Endotoxin and Immune System**

LPS is a very potent stimulator of the cells of the immune system, including monocyte/macrophages, B cells, polymorph nuclear cells (PMNs), and vascular endothelial cells (EC). It is the ability of host cells to respond to LPS by producing a wide range of immunological and pharmacological mediators that results in Gram-negative septic shock. The effects (including lethal toxicity, pyrogenicity, activation of B cells, and induction of cytokines) can all be attributed directly to the lipid A moiety of the LPS.4

Endotoxins also cause an inflammatory response of the respiratory tract. The response is mediated by the proinflammatory cytokines IL-1, IL-6, IL-8, and TNF-α and gives rise to general symptoms (fever, headache, malaise), respiratory symptoms (tightness of chest, dry cough), and lung function decrements.5

Ever since the pharmaceutical industry began manufacturing injectables, endotoxin detection tests have been an absolute necessity. Today, LAL testing is used in the medical device, pharmaceutical, dialysis and bioresearch industries to routinely test products.

**Endotoxin Removal**

Removal of endotoxin is one of the most difficult downstream processes during protein purification. Many commercially available products are unable to remove endotoxin sufficiently, or require time consuming incubation steps. In many cases, complete endotoxin removal is only achieved with massive substrate loss.

Common late downstream protein solutions are concentrated between 0.1 – 50 mg/mL. Reduction or removal of endotoxin to less than 1 ng/mg (10 EU/mg) is a very difficult task. By using selective sorbents, endotoxin removal from proteins has clear limits. Only methods with the highest endotoxin removal capacity combined with excellent recovery rates (of the target substance) are reasonable and acceptable. To meet these most challenging requirements, EndoTrap was developed.

**Characteristics of EndoTrap®**

EndoTrap is an affinity matrix for the efficient removal of bacterial endotoxins from solutions. EndoTrap can be employed both in batch or chromatography mode. EndoTrap has been developed for the removal of endotoxins from aqueous solutions containing low or high molecular weight substances. Frequently, endotoxin removal from protein solutions is insufficient with standard methods including ultrafiltration, ion exchange chromatography, or two phase extraction.
Principles of EndoTrap®-Based Affinity Chromatography:

A: Principle of EndoTrap®

1. Application of sample
   Endotoxin – contaminated proteins and aqueous solutions are applied to column

2. Removal of Endotoxin
   Unwanted Endotoxin is captured / proteins can be collected

3. Regeneration of ligand
   Regeneration of ligand by using appropriate buffer

B: Binding and elution profile of sample

Key:
- Endotoxin/LPS
- Protein of interest
- Ligand attached to agarose beads
The protocol of EndoTrap is user-friendly, yields rapid results as it is designed as a flow-through system. No training or special equipment is required. EndoTrap is applicable also for the downstream process as it is also available as a 50% slurry. The EndoTrap resin can be applied on fully automated liquid chromatography systems. Therefore EndoTrap connects the research and the production field.

EndoTrap is available as EndoTrap Blue and EndoTrap Red depending on your desired applications. Both members of the EndoTrap-family have similar endotoxin removal characteristics and differ in their buffer requirements.

### Following Characteristics Underline the Outstanding Performance of EndoTrap:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast</td>
<td>No incubation step – real flow-through system</td>
</tr>
<tr>
<td>High recovery rate</td>
<td>Minimized sample loss</td>
</tr>
<tr>
<td>LPS variability</td>
<td>Endotoxin removal from broad range of bacteria strains</td>
</tr>
<tr>
<td>Re-usable / Columns can be regenerated</td>
<td>Re-usable at least three times without any loss of endotoxin removal efficiency; - regeneration buffer included and does not contain Deoxycholate (DOC)**!</td>
</tr>
<tr>
<td>Temperature stability</td>
<td>Regular use in range between 4°C and room temperature</td>
</tr>
<tr>
<td>Kit includes</td>
<td>Ready to use columns, regeneration and equilibration buffers</td>
</tr>
</tbody>
</table>

### Customer Applications

#### Cell Culture
- immune modulation e.g. T cell or B cell stimulation
- immune suppression e.g. dendritic cells
- apoptosis e.g. primary endothelial cells
- pro-inflammatory responses
- TLR (toll-like receptors)

#### Animal Models
- proliferation assays
- vaccine e.g. injection into mouse and monkey
- immune stimulation e.g. HIV envelope
- sepsis

#### Research Topics

**(Sodium) Deoxycholate would have cytotoxic effects on cell culture and also influence the cell growth and the morphology of the cells. It is also reported that deoxycholate can induce DNA damage.
LPS Removal from Different Aqueous Solutions with EndoTrap®

In general, a range of substances can be applied onto the column, limited only by viscosity and the ability to pass through the column. EndoTrap can be used with substances like proteins, peptides and antibodies, even with high molecular weights. Protein concentrations above 50 mg/mL have successfully been applied onto the column. However, we recommend a working concentration of 1-10 mg/mL. EndoTrap removes endotoxin from proteins with isoelectric points (pI) from 5 to 9.

EndoTrap is based on proteinaceous affinity ligand derived from a bacteriophage, which binds to endotoxins with a high affinity constant. EndoTrap ligands are not antibodies, Polymyxin B or synthetic peptides.

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.

EndoTrap is an excellent example of a high performance, reliable and easy to use system:
- It reduces endotoxins to less than 1 ng/mg
- Is very selective for endotoxins combined with extremely high binding capacity
- Shows excellent recovery rates of the target substance
- It is based on a new proprietary affinity protein
- It is a flow through system, which does not require time consuming incubation steps
- Re-usable, regeneration substance is not Deoxycholate
- All buffers included with kits

Cambrex is the industry leader in sales and innovations of endotoxin detection products, services and software. Cambrex is a global, diversified life science company dedicated to providing innovative products and services to accelerate drug discovery, development, and manufacturing processes for customers focused on health and the prevention of disease.

With EndoTrap, the scientist can now remove endotoxin contamination quickly without expensive training or costly equipment.

The QCL-1000 Chromogenic End-point Assay is appropriate for any laboratory looking for a rapid turnaround. This 16-minute quantitative end-point assay measures endotoxin levels photometrically and has a sensitivity range of 0.1 EU/mL to 1.0 EU/mL.

Users new to the QCL-1000 assay may experience potential problems that may yield unexpected results. This article is intended to provide the technician with valuable tips to improve technique and yield good assay results:

**Importance of Glass Tubes and Vortexing**

It is very critical that pyrogen-free glass tubes are used when making control standard and sample dilutions. This is important because endotoxin may adhere to plastic tubes and cause variability in the amount of endotoxin detected in a sample (or standard). It is also recommended that glass tubes be used for storing stock reagents and samples. When performing the test tube method, reaction tubes should be made of glass.

Cambrex offers Pyrogen-free Test Tubes for dilution, part code N207 and glass Pyrogen-free reaction tubes (N201 and N205).

Adequate mixing of the endotoxin and standards by vortexing will improve the linearity of the assay. It will ensure that the endotoxin does not form aggregates in solution, greatly improving test reactivity. The control standard endotoxin must be vortexed for a minimum of 15 minutes after initial reconstitution and each use thereafter. Each endotoxin standard must be vortexed a minimum of one minute before use.

**Heat Block Consistency**

When using a heat block, it is important that the heat be maintained across the heat block (from tube to tube or across the plate) at a consistent 37±1°C. This is significant because the rapid rate of the LAL reaction requires rapid and uniform heat transfer. Temperature should be monitored with a calibrated thermometer and checked before each use. Using an aluminum block plate holder can correct problems associated with inconsistent heating and varying temperature edge affects on the plate itself. (Note that it is not recommended to use an incubator, warm room or other inconsistent heat sources.)

A Ground Aluminum Block (heat block adapter) can be purchased from Cambrex, Part Number 25-038.

**1 EU/mL Standard Prep**

Another potential pitfall is encountered when making the 1 EU/mL standard. The Certificate of Analysis (COA) is an essential reference because it states the actual reconstituted potency of the stock vial. This potency can vary from lot to lot, and it is important to reference the correct COA for the lot you are currently using. The endotoxin must be reconstituted with 1 mL of LAL Reagent Water and vortexed for 15 minutes. To make the 1 EU/mL standard, the volume of water to be used for the dilution should be determined using the reconstituted potency and following formula: (X-1)/10 mL (where X is the concentration value found on the COA).

Example: (24-1)/10 mL or 2.3 mL

Then, 0.1 mL of the stock CSE solution is added to 2.3 mL of water. This standard is vortexed for 1 minute and used to make all subsequent standard dilutions.

**Potential Endotoxin Contaminants**

At times, you may have an unexpected false-positive result. This is often a result of endotoxin contamination. Probable sources of contamination include dilution and reaction tubes, pipette tips, serological pipettes, and water used for reconstitution and dilution. Check with the manufacturer of all accessory materials to verify that they have been tested for endotoxin at a level below the sensitivity of your kit. For your convenience, Cambrex offers a line of accessory materials that meet this requirement. Please see the following article and the Cambrex Bio Science catalog for more information.
Screening Tips and Microplates for LAL Testing

By Maribeth Donovan Janke, Ph.D.

Regardless of which endotoxin detection method you are using in the laboratory, you are no doubt using plastic accessories at some point in the procedure. For a gel clot assay, you may be using plastic pipette tips to dispense samples and LAL reagent into the glass reaction tubes. If you are using a kinetic method to test for endotoxin, you more than likely are running the assay in a disposable plastic microplate.

The package inserts provided by the LAL manufacturers caution that care must be taken to avoid microbiological or endotoxin contamination of the sample to be tested or the LAL reagent. As an example, the Cambrex Kinetic-QCL® package insert states: “All materials coming in contact with the specimen or test reagents must be endotoxin-free.” Glassware easily is rendered endotoxin-free by heating at 250°C for 30 minutes. But what do you do about the plastic accessories used in the test?

The Kinetic-QCL insert also states: “From experience, most sterile, individually-wrapped, plastic pipettes and pipette tips are endotoxin-free. However, these materials should be tested before regular use.” Testing of plastic accessories is not just a suggestion from Cambrex; it is also a requirement of the United States Pharmacopeia (USP). In the USP Chapter <161>, Bacterial Endotoxins Test (BET) it states: “if employing plastic apparatus, such as microplates and pipet tips for automatic pipetters, use only that which has been shown to be free of detectable endotoxin and not to interfere with the test.” Portions of the USP BET were harmonized in 2000 with the European and/or Japanese Pharmacopoeias. All three Pharmacopoeias are in agreement on this statement. Therefore, testing of plasticware is not just a requirement in the United States; it is a global requirement.

Procedures should be established for testing the plastic items used in LAL testing. Some suggestions for pipette tips and microplates will be offered here.

One method for developing a testing protocol for pipette tips and microplates is to borrow the formula for testing medical devices. USP Chapter <161> Transfusion and Infusion Assemblies and Similar Medical Devices describes a procedure for extracting endotoxin from a number of devices into a rinse or soak solution (typically LAL Reagent Water) and testing that solution for endotoxin. The volume of the solution may vary depending on the size of the device. For instance, more volume will be needed to soak 10 microplates than it will to soak ten 200 µL pipette tips. The soak solution should be in contact with the device sampling for 1 hour at room temperature. If plates or tips are soaked or rinsed individually, it is allowable to pool the solutions prior to testing with an LAL method. The volume of the pooled solution will be taken into account when determining whether or not the lot of tips or microplates has passed the endotoxin test.

From USP Chapter <161>, the endotoxin limit for the solution is equal to

\[
\text{Endotoxin Limit} = \frac{K \times N}{V}
\]

where K is the endotoxin limit per device, N is the number of devices tested and V is the total solution volume. The acceptable endotoxin limit for a medical device is 20 EU/device. Those devices coming into contact with the cerebrospinal fluid have an endotoxin limit of 2.15 EU/device. However, applying either of these limits to a pipette tip or a microplate well would not be appropriate. As you would prefer “endotoxin-free”, zero EU/device is what you want, but you cannot test for zero. You will have to decide what the appropriate endotoxin limit for each tip or microplate well should be, for your use.
As you set the “device” endotoxin limit for plastic accessories, keep in mind that you may be testing a pooled extraction solution. If you set a limit too low, your solution volume may dilute the solution endotoxin limit below the detection level of any current LAL method. If you set a limit too high, a passing lot may contain just enough endotoxin to cause positive LAL reactions (“hot wells”) during the routine testing of products. Here are two examples.

When you use a pipette tip for adding Kinetic-QCL reagent to your reaction vessel, 100 µL (or 0.1 mL) of the reagent is dispensed. This reagent is sensitive to 0.005 EU/mL or 0.0005 EU/tip (calculated by 0.005 EU/mL x 0.1 mL/tip = 0.0005 EU/tip). If you set your tip endotoxin limit at 0.0005 EU/tip and test 10 tips by soaking in 100 mL of water, then the calculated endotoxin limit for the solution is (0.0005 EU/tip x 10 tips)/100 mL or 0.00005 EU/mL. Since this value is below the sensitivity of the Kinetic-QCL method, negative results will be meaningless. You will not catch anything that falls between 0.00005 EU/mL and the detection limit of 0.005 EU/mL for the Kinetic-QCL kinetic chromogenic assay. Some tips may have detectable endotoxin levels that could cause problems during your routine product testing.

Using a multichannel pipettor will allow for quick rinsing of several pipette tips at the same time. With this in mind, the rinse volume should be set at a level that will fit in a reagent reservoir and adequately fill each of the 8 tips with each rinse cycle. Although only 100 µL is dispensed typically in an LAL test, rinsing as much of the interior surface of the tip as possible is preferable for the endotoxin screening protocol. So, for a 200 µL pipette tip, the rinse volume per tip should be 200 µL. Eight (8) pipette tips by 200 µL per tip is 1.6 mL of rinse water. An additional amount should be added so that there is enough liquid in the reagent reservoir to fill the tips equally and still have some liquid remaining. A total of 2.5 mL should be acceptable for a typical reagent reservoir.

An acceptable endotoxin limit for the rinse solution should be chosen. This will be the pass/fail acceptance limit for the test. If 0.02 EU/mL is chosen, this means that if the tip rinse solution contains greater than 0.02 EU/mL, then the lot of tips being tested fails.

With the endotoxin limit per tip (K), the total rinse solution volume (V) and an acceptable endotoxin limit for the rinse solution, there is one variable missing for the test: the number of tips to test (N). The formula listed above for medical device testing is (K x N)/V equals the solution release limit. Rearranging the equation to solve for N yields:

\[ N = \frac{\text{Endotoxin Release Limit} \times V}{K} \]

Inserting the values discussed above, N equals (0.02 EU/mL x 2.5 mL)/0.0005 EU/tip. The number of tips to be tested equals 100 tips.

So the procedure could be as follows. Fill a reagent reservoir with 2.5 mL of LAL Reagent Water. Using an 8-channel multipipettor, attach 8 tips and wash up and down with 200 µL per tip several times into the reservoir. Discard the tips and pick up 8 fresh ones. Wash these with the same 2.5 mL in the reservoir. Keep repeating until 100 tips have been rinsed into the reservoir (the last set will contain 4 tips). Test the solution in the reagent reservoir to determine the endotoxin content, including a positive product control. If the endotoxin content is less than the
0.02 EU/mL limit, then the lot of tips passes. If the tested content is above 0.02 EU/mL, then the lot of tips fails. A passing test result indicates that the tips contain less than 0.0005 EU/tip.

**A Procedure for Screening 96-well Microplates for Endotoxin Testing**

Just as 0.0005 EU/tip is desired to avoid an apparent “hot well” due to a “hot” tip, a similar endotoxin limit should be established for the microplates used in LAL testing. However, instead of attempting to soak a number of plates, running a procedure that mimics how the microplates are used in the assay is preferred, by screening the microplates by testing individual wells on a number of plates. If you are familiar with running a Uniformity Assay, the screening test is similar. But, instead of placing a single endotoxin standard in each well of the 96 wells and running the assay to determine the reaction time % CV across the plate for that standard, the screening test just uses LAL Reagent Water in a majority of the wells. A few wells are used for positive controls, containing the lowest standard detected by the LAL assay of choice. The test involves determining whether or not the reaction times of the water-only wells are slower than the positive control wells, thus indicating that the endotoxin content of the water-only wells are below that level. For a Kinetic-QCL assay, 0.005 EU/mL is the lowest standard. As 0.1 mL is tested, the resulting endotoxin level is 0.0005 EU/well. So the overall test results may indicate that the endotoxin content of the tested microplate is less than 0.0005 EU/well.

The following procedure could be used to screen microplates. For each new lot of 96-well microplates, a specified number of plates are chosen at random for testing. One hundred (100) µL of LAL Reagent Water is placed in all of the wells on each plate. To 4 of the wells on each plate, a quantity of endotoxin should be added varying the location among the plates. For instance plate 1 may have the 4 positive control wells in positions A1, B2, C3 and D4, while plate 2 has the control wells in E9, F10, G11, and H12. A 10 µL aliquot of a single endotoxin standard solution should be placed in the control wells. For the Kinetic-QCL test, 10 µL of the 0.05 EU/mL standard will be placed in the control wells, yielding 0.005 EU/mL in each. The 0.05 EU/mL standard is made by making dilutions of a concentrated endotoxin standard.

The microplate containing the LAL Reagent Water and the positive control wells is placed in the incubating microplate reader. If using the Cambrex WinKQCL® software, a Uniformity Test can be started directly from the software. Once initiated, the plate will incubate at 37°C for 10 minutes. The software will prompt you as to when to add the LAL reagent and to start the assay. At the end of the assay, a Uniformity Test Report showing the reaction times for each well can be printed. This will be repeated for all of the test microplates.

When reviewing the results, both the positive control wells and the water-only wells should be examined. Calculate the % CV for the positive control wells and verify that it is < 10%. This confirms that there is consistency among the control wells. Next, note the longest (highest) reaction time for the 4 positive control wells. Compare this time with the reaction times for the 92 wells that only contained LAL Reagent Water. Any of the water-only wells with a shorter reaction time (lower number) than the longest positive control well, are marked as positive.

After testing the microplates, the number of positive wells (among the water-only wells) per microplate and per batch of all the plates sampled should be examined. An acceptable number of positives should be established, based on a statistical confidence level, say 95% of a specified failure rate, and the test results compared to that acceptable number. For instance, when testing a sample set of 6 microplates, the per individual plate acceptable level could be set at “no more than 4” positive wells, while the per batch of 6 plates level could be set at “no more than 11”. Any lot that meets the % CV specification for the positive controls and both the individual plate and the batch of plate levels can be passed for use in LAL testing.
The methods described here are suggestions for the routine testing of new lots of tips and microplates. When screening items from a new vendor, a more extensive validation protocol may be required that is similar to what is done for a new product: for instance, the same procedure on 3 different production lots. Not all plastic is the same. There may be differences from vendor to vendor as well as lot to lot variability from a single vendor.

Setting endotoxin limits at 0.0005 EU/tip and 0.0005 EU/well can be testable. Lots which meet these limits will provide the end-user with confidence that the plasticware used for LAL testing will not interfere with the routine testing of products. Each end-user will have to determine what procedures and endotoxin limits are acceptable to them.

**Save Time with Pre-Screened Plasticware**

An alternative to in-house testing of plasticware is to purchase tips and plates that are already tested for endotoxin content. Pre-screened pipette tips and 96-well microplates are available from Cambrex (Table 1). Certificates indicate that the lot was tested and shown to be less than a particular endotoxin content. For instance, the Certificate of Analysis for a lot of LAL Reagent Grade Multi-Well Plates lists the endotoxin content as less than 0.0005 EU/well.

Meeting the recommendation of the LAL manufacturers and the requirement of the USP BET does not need to be overwhelming. Testing procedures can be devised to test plasticware at the low levels of sensitivity available with the current LAL methods. End-users can also purchase pre-screened plasticware that has been tested at low levels. Limiting “hot” tips or wells will keep retesting to a minimum, reducing the cost of QC testing and increasing your confidence in your routine testing results. Whether you test your plasticware or purchase them pre-screened, you should be confident the plastic accessories being used are as endotoxin-free as can be determined.

**References:**
2. Bacterial Endotoxins Test, Chapter <85>, USP 28, NF 23, Supplement 1, 2005, United States Pharmacopeia, Rockville, MD.
3. Transfusion and Infusion Assemblies and Similar Medical Devices, Chapter <161>, USP 28, NF 23, Supplement 1, 2005, United States Pharmacopeia, Rockville, MD.

**Table 1. Pre-Screened Plastic Accessories Available from Cambrex**

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Cat. No.</th>
<th>Size</th>
<th>Quantity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eppendorf Biopur®</td>
<td>25-415</td>
<td>200 µL</td>
<td>For all 3 sizes:</td>
<td>---</td>
</tr>
<tr>
<td>Pipette Tips</td>
<td>25-416</td>
<td>300 µL</td>
<td>5 trays per package</td>
<td>Only for multichannel pipettors</td>
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<tr>
<td></td>
<td>25-417</td>
<td>1000 µL</td>
<td>96 tips per tray</td>
<td>---</td>
</tr>
<tr>
<td>LAL Reagent Grade Plates</td>
<td>25-340</td>
<td>---</td>
<td>50 plates per case</td>
<td>96-well plates, &lt;0.0005 EU/well</td>
</tr>
</tbody>
</table>

**What’s New**

**EndoTrap® System for Removing Endotoxin**

Cambrex Bio Science Walkersville, Inc., the leading provider of endotoxin detection products, services and software, is pleased to offer the EndoTrap System. EndoTrap is the new affinity matrix for the efficient removal of bacterial endotoxin from aqueous solutions. EndoTrap consists of a very high-affinity ligand material derived from bacteriophages, linked to sepharose beads, to create an easy-to-use flow through column with maximum endotoxin removal capability.

Currently available endotoxin removal methods frequently suffer from excessive loss of the protein during the process, making purification an expensive proposition. EndoTrap efficiently binds to endotoxin even at very low concentrations and minimizes binding of the target protein due to the high ligand specificity. Protein recovery in the range of 92-99% is possible in most cases. EndoTrap is ideal for removal of endotoxin in cell culture.
## EndoTrap® Blue

<table>
<thead>
<tr>
<th>Product Family</th>
<th>Description</th>
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<tbody>
<tr>
<td>83-000U EndoTrap Blue 1/1</td>
<td>1 x 1 mL column, 125 mL Equilibration Buffer, 125 mL Regeneration Buffer</td>
</tr>
<tr>
<td>83-001U EndoTrap Blue 5/1</td>
<td>5 x 1 mL column, 250 mL Equilibration Buffer, 125 mL Regeneration Buffer</td>
</tr>
<tr>
<td>83-002U EndoTrap Blue 10</td>
<td>20 mL (50% slurry) resin, 250 mL Equilibration Buffer, 250 mL Regeneration Buffer</td>
</tr>
<tr>
<td>83-003U EndoTrap Blue 50</td>
<td>100 mL (50% slurry) resin, 125 mL 10x Equilibration Buffer, 125 mL 10x Regeneration Buffer</td>
</tr>
<tr>
<td>83-004U EndoTrap Blue 100</td>
<td>200 mL (50% slurry) resin, 250 mL 10x Equilibration Buffer, 250 mL 10x Regeneration Buffer</td>
</tr>
<tr>
<td>83-005U EndoTrap Blue Bulk</td>
<td>Bulk resin for industrial applications</td>
</tr>
<tr>
<td>83-006U Equilibration Buffer for EndoTrap Blue</td>
<td>125 mL Equilibration Buffer “Blue”; endotoxin concentration &lt; 0.02 EU/mL</td>
</tr>
<tr>
<td>83-007U Regeneration Buffer for EndoTrap Blue</td>
<td>125 mL Regeneration Buffer “Blue”; endotoxin concentration &lt; 0.02 EU/mL</td>
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</tbody>
</table>

## EndoTrap® Red

<table>
<thead>
<tr>
<th>Product Family</th>
<th>Description</th>
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<tbody>
<tr>
<td>83-008U EndoTrap Red 1/1</td>
<td>1 x 1 mL column, 125 mL Equilibration Buffer, 125 mL Regeneration Buffer</td>
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<tr>
<td>83-009U EndoTrap Red 5/1</td>
<td>5 x 1 mL column, 250 mL Equilibration Buffer, 125 mL Regeneration Buffer</td>
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<td>83-010U EndoTrap Red 10</td>
<td>20 mL (50% slurry) resin, 250 mL Equilibration Buffer, 250 mL Regeneration Buffer</td>
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<td>83-011U EndoTrap Red 50</td>
<td>100 mL (50% slurry) resin, 125 mL 10x Equilibration Buffer, 125 mL 10x Regeneration Buffer</td>
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<tr>
<td>83-012U EndoTrap Red 100</td>
<td>200 mL (50% slurry) resin, 250 mL 10x Equilibration Buffer, 250 mL 10x Regeneration Buffer</td>
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<tr>
<td>83-013U EndoTrap Red Bulk</td>
<td>Bulk resin for industrial applications</td>
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<tr>
<td>83-014U Equilibration Buffer for EndoTrap Red</td>
<td>125 mL Equilibration Buffer “Red”; endotoxin concentration &lt; 0.02 EU/mL</td>
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<tr>
<td>83-015U Regeneration Buffer for EndoTrap Red</td>
<td>125 mL Regeneration Buffer “Red”; endotoxin concentration &lt; 0.02 EU/mL</td>
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<table>
<thead>
<tr>
<th>Product Family</th>
<th>Description</th>
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<tbody>
<tr>
<td>83-100 Empty Columns, Large</td>
<td>for 2 – 10 mL resin, pack of 5 columns</td>
</tr>
<tr>
<td>83-101 Empty Columns, Small</td>
<td>for 0.2 – 2 mL resin, pack of 5 columns</td>
</tr>
</tbody>
</table>
supernatants, in-process material, and protein products.

EndoTrap is available in two versions: EndoTrap Blue and EndoTrap Red. Both versions have similar capabilities for endotoxin removal and selection is based on the ionic strength and pH range of the sample solution. The product is shipped in ready-to-use columns. After removing the column cap, draining the storage solution and activating the column, the sample is applied. As the solution drains, the product is endotoxin-free. Afterwards, the endotoxin can be removed and the columns reused.

**Cambrex Opens a New Subsidiary in Spain**

To better serve our customers, Cambrex Bio Science has opened a new subsidiary in Spain – Cambrex Iberia Products, S.r.l., phone: 34 90 253 1366.

This new subsidiary will be tasked with serving Endotoxin Detection customers as well as Bioresearch and Cell Therapy customers.

Salvador Garrido will be heading up the operation assisted by Susana Martinez, Tania Alba and Ana Nunez.

(Susana Martinez: Sales

(Below, left to right)

Tania Alba: Customer Service
Salvador Garrido: Managing Director, Sales
Ana Nunez: Purchasing Dept.)