EtoxiClear™: A new adsorbent for the efficient removal of endotoxin from biopharmaceuticals

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Introduction
Endotoxin or lipopolysaccharide (LPS) (Figure 1) are highly toxic components of the cell wall of Gram-negative bacteria and are often present in significant amounts in bacterial cell culture expression systems such as E. coli.

The bi-tert-amine ligand, attached to ProMetic Biosciences Ltd (PML’s) proprietary base-matrix – Puradex®, binds in a spatially selective and optimal manner to the LPS molecule with a binding capacity for endotoxin in excess of 1,000 EU/g of adsorbent in a flow through chromatography mode (5 minute residence time).

A number of biopolymers with different isoelectric points have been used to demonstrate efficient protein recovery and clearance of residual endotoxin across the pH range. Protein recoveries in excess of 95% are achievable with endotoxin clearance to below the acceptable level of 0.1 EU/mg protein.

Binding Capacity
EtoxiClear™ has a high dynamic binding capacity for endotoxin in the presence of a wide range of biological buffers. The dynamic binding capacity is shown in Table 1.

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<th>Product Comparison</th>
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<tbody>
<tr>
<td>EtoxiClear™</td>
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<tr>
<td>Endotoxin Binding Capacity</td>
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<td>Endotoxin (EU) [mL of Endotoxin]</td>
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<td>EtoxiClear™</td>
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<td>≤1,000,000 EU/mg*</td>
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* Linear flow rate 1200/L/hr, 5 minute residence time

Product Recovery vs Endotoxin Concentration
IgG protein solutions (~5.5 mg/mL), containing either low (10.0 EU/mL) or high (2300 EU/mL) levels of endotoxin, were loaded onto EtoxiClear™ disposable columns (5 mL column volume [CV]). Each column was loaded with similar total amounts of endotoxin (~10,000 EU) and protein recoveries were measured (Table 3).

Protein Binding
EtoxiClear™ has low protein binding and a wide range of proteins can be processed independently of their isoelectric point achieving high protein recoveries. Figure 3 indicates that typically 90% recovery is achieved for various model proteins spanning the pH spectrum.

Effect of protein concentration on endotoxin removal and protein recovery
Increasing F(ab)2 concentration (from 0.8 to 8 mg/mL) resulted in increased target protein recovery (from 80% to 95%) respectively when loading 24,000 EU/mL of endotoxin onto EtoxiClear™ at neutral pH. Results indicate that increasing protein concentration has no significant effect on the level of endotoxin clearance obtained.

Endotoxin removal from purified antibody fragment
EtoxiClear™ was used to remove residual endotoxin from an antibody fragment from E. coli culture partially purified using Fabsorbent™ F1P HF as a capture step (Table 4). Clarified cell lysate was loaded onto Fabsorbent™ F1P HF and the (F(ab)2), fragment eluted at pH 5.5. The resulting elution fraction was loaded directly onto EtoxiClear™.

Buffer pH
EtoxiClear™ can operate in acidic to neutral conditions (pH 4.0 to pH 8.0) without a reduction in endotoxin clearance and typically maintaining >90% recovery of various proteins (up to 1 mg/mL).

Endotoxin removal from proteins expressed in E. coli
Two different proteins, produced in E. coli (post initial capture step), were applied to EtoxiClear™ at neutral pH. Results presented in Table 5 below show high protein recovery and significant endotoxin clearance.

Determination of β-D-Glucan Interference
There are many commercially available endotoxin detection tests/kits to determine endotoxin clearance from protein solutions. However, a chromogenic based (LAL) test is used, it is recommended to include Glucashield® buffer to render the reagent insensitive to (1-3)-β-D-Glucan interference which may be present in the sample.

Inhibition of the β-D-glucan interference allows for more sensitive and more accurate determination of endotoxin removal comparable to the analysis performed externally at the Lanza Bioscience laboratory.

Conclusions
• EtoxiClear™ provides superior endotoxin removal from a wide range of proteins across the pl spectrum, with recoveries that can be in excess of 95%. In a range of conditions from acidic to neutral pH.
• EtoxiClear™ has a high capacity for endotoxin and binds ≤1,000,000 EU/mL of adsorbent loading at 1200/L/hr, 5 minute residence time.
• EtoxiClear™ shows improved endotoxin clearance and protein recovery in comparison to other commercially available endotoxin removal products.
• EtoxiClear™ gives excellent endotoxin clearance (~1.0 EU/mL) and high protein recoveries for protein solutions containing either low or high starting concentrations of endotoxin.
• Protein recoveries can be improved by increasing the target protein concentration without any impact on endotoxin/binding.
• Following the capture and partial purification of an antibody fragment, EtoxiClear™ provided a 4 log reduction of endotoxin across the process.
• Endotoxin clearance of ~2 log from purified proteins expressed in E. coli was obtained using EtoxiClear™.
• The introduction of Glucashield® buffer to remove interference by β-D-Glucans improved the sensitivity of the chromogenic assay and provided a more accurate determination of endotoxin clearance.
• Overall, EtoxiClear™ is ideally suited for use in process development applications or final polishing steps used during cGMP manufacturing of biopharmaceuticals.

References
1. http://www.lanzabiotechnology.co.uk/ 
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