TOYOPEARL® NH_2-750F
Flow-through Removal of Endotoxin

Endotoxins are remnants of bacterial cell walls that may contaminate drug products and cause an immunogenic response. They are often referred to as "pyrogens" due to their fever-inducing effects. Endotoxins may be found in drug products either due to contamination from host cells used to produce a drug product in a bacterial expression system or due to adventitious bacterial contamination in non-microbial products. Thus, endotoxin clearance is a requirement of downstream processing of biologics, especially those derived from microbial expression systems that contain endogenous host cell endotoxin. In this study, we evaluate the ability of TOYOPEARL NH_2-750F for the removal of endotoxins by anion exchange chromatography.

Introduction

TOYOPEARL NH_2-750F, a salt-tolerant anion exchange resin for process scale applications, is based on the TOYOPEARL HW-75F size exclusion resin functionalized with primary amine groups. *Table 1* lists the properties of the TOYOPEARL NH_2-750F resin.

Table 1. Properties of TOYOPEARL NH_2-750F

	TOYOPEARL NH_2-750F
Particle size (µm)	30-60
Pore size (nm)	> 100
Ion Exchange capacity (eq/L resin)	0.07 – 0.13
SBC (g/L resin)	≥ 70

TOYOPEARL NH_2-750F resin is ideal for the intermediate purification of mAbs and other proteins where aggregates and other negatively charged impurities, such as DNA and endotoxins, are removed from the target of interest without having to dilute or buffer exchange the product prior to loading.

The data presented here demonstrate the capabilities of TOYOPEARL NH_2-750F to remove endotoxin in a flow-through chromatography process.

Experimental Conditions

An ÄKTA® system was flushed with 75 mL of 0.1 mol/L phosphoric acid, followed by 75 mL of E-pure water. The system was then flushed with 0.5 mol/L sodium hydroxide and incubated for a minimum of 1 hour at ambient temperature. Following the incubation, the system was flushed with 75 mL of E-pure water, and then with 20 mmol/L Tris base, pH 7.4, until a stable conductivity and pH baseline was noted. Adequate system cleaning was verified by Limulus amebocyte lysate (LAL) assay.

A TOYOPEARL NH_2-750F MiniChrom column (8 mm ID × 10 cm) was cleaned-in-place with 5 CV of 0.5 mol/L sodium hydroxide with a 15 minute contact time. The column was regenerated with 2 CV of 20 mmol/L Tris base, 1 mol/L NaCl, pH 7.4, and then equilibrated with 20 mmol/L Tris base, pH 7.4, until a stable conductivity and pH baseline was obtained (ca. 3 CV).

All solutions used in this experiment were tested for background endotoxin levels by LAL assay and were found to be endotoxin free, though the strip buffer did show some trace amounts of endotoxin with the LAL assay. Therefore the strip sample was diluted with endotoxin-free water prior to testing.

A 5 mg aliquot of lipopolysaccharide (LPS) was suspended in 1.0 mL endotoxin-free water with vigorous mixing for 5 minutes. Dilutions of the LPS solution were tested for endotoxin activity by LAL assay. The endotoxin stock solution was protected from light and refrigerated until use.

The column was equilibrated with 20 mmol/L Tris base, pH 7.4, until a stable conductivity and pH baseline was obtained. A 50 mL sample of equilibration buffer (20 mmol/L Tris base, pH 7.4) was spiked with standardized LPS solution to a final concentration of approximately 100,000 EU/mL. The column was then loaded with spiked equilibration buffer and 2 CV (10 mL) flow-through fractions were collected. Following loading, the column was washed with 3 CV of equilibration buffer and stripped with 3 CV of a high-salt buffer (20 mmol/L Tris base, 1 mol/L NaCl, pH 7.4). Fractions were collected for both wash and strip steps. The column was cleaned-in-place, as described previously, prior to subsequent runs or storage in 20% ethanol.

The load material, flow-through, wash, and strip fractions were all tested for endotoxin by LAL assay.

Results

For the endotoxin clearance, a solution of *E. coli* lipopolysaccharide was prepared in water. A 5 mg/mL LPS solution gave an endotoxin activity of 5.6×10^7 EU/mL when tested at a 1:1 x 10^8 dilution. The column loading material was prepared to have an endotoxin content of approximately 100,000 EU/mL in column equilibration buffer. A direct assay of this solution gave a starting concentration of 89,000 EU/mL with a total load of 4,450,000 EU (89,000 EU/ mL × 50 mL).

To calculate the endotoxin clearance, the following method was used:

Sample endotoxin concentration (C_i) was determined to be 89,000 EU/mL as stated above. The log endotoxin content (L_i) for the load sample was determined from the sample endotoxin concentration and the load volume (V_i):

$$L_i = \log_{10} C_i + \log_{10} V_i$$

For the load sample:

$$L_{Load} = \log_{10} C_{Load} + \log_{10} V_{Load} = \log_{10} 89{,}000\ \text{EU/mL} + \log_{10} 50.0\ mL = 6.65$$

Endotoxin clearance for a given fraction (A_i) was determined by subtraction.

$$A_i = L_{Load} - L_i$$

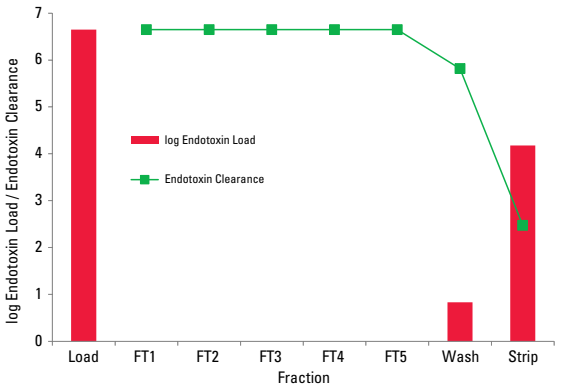
For the wash sample:

$$A_{Wash} = L_{Load} - L_{Wash} = 6.65 - 0.83 = 5.82$$

As can be seen in *Figure 1*, there was some minor breakthrough of endotoxin during the wash phase, and the log reduction value for this fraction was 5.82. Please note that this represents a breakthrough of less than 0.0002% of endotoxin from the original load material.

The endotoxin concentration of the flow-through fractions was less than the limit of detection for the assay (0.1 EU/mL); therefore, the minimum log reduction value for each flow-through fraction was 6.7. *Figure 1* shows a graphical representation of the log endotoxin clearance for each step in the process.

Figure 1. Endotoxin clearance summary



Resin:	TOYOPEARL NH_2-750F
Column:	MiniChrom, 8.0 mm ID × 10.0 cm (5.0 mL)
Mobile phase:	A: 20 mmol/L Tris base, pH 7.4
	B: 20 mmol/L Tris base, 1 mol/L sodium chloride, pH 7.4
Flow rate:	150 cm/hr (1.25 mL/min, 4 min residence time)
Detection:	LAL endotoxin assay
Temperature:	ambient
Injection vol.:	5 × 2 CV (10 mL) injections,
	10 CV (50 mL) total
Sample:	89,000 EU/mL LPS in equilibration buffer
Instrument:	ÄKTA Explorer 100

Conclusions

TOYOPEARL NH_2-750F is a very effective anion exchange resin for the removal of endotoxin in a flow-through chromatography mode. This experiment shows that TOYOPEARL NH_2-750F is capable of reducing endotoxin in a sample to levels below the limit of detection for an LAL assay, in this instance greater than 6.7 logs.

TOSOH BIOSCIENCE

TOSOH BIOSCIENCE LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Tel: 800-366-4875
email: info.tbl@tosoh.com
www.tosohbioscience.com

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