Affi-Prep® Polymyxin Support

ENDOTOXIN REMOVAL FOR THE RESEARCH AND PROCESS SCALE LABORATORY

Endotoxins, pyrogenic lipopolysaccharides of gram-negative bacteria, are widespread contaminants of aqueous and physiological solutions. The removal of endotoxins from solutions intended for biological applications is crucial, especially for *in vivo* applications, because even nanogram per milliliter concentrations of endotoxins have been shown to produce pyrogenic and shock reactions in humans and animals, and experimental artifacts in cell culture studies.

As therapeutic and pharmacologically important products are developed in the biotechnology and pharmaceutical industries, the need for efficient chromatographic supports for endotoxin removal has become apparent. Besides removing endotoxins with high efficiency and selectivity, these supports should be able to stand the harsh treatments necessary for adequate sanitation before use. They should also be macroporous, to provide a high surface area, yet rigid enough to satisfy the scale-up requirements of industrial production.

Due to its high affinity for the lipid A moiety of endotoxins,[1,2] polymyxin B is finding increasing application in the removal of endotoxin from preparations intended for parenteral use. Although several polymyxin-based matrices are currently available, none of them fill all of the above requirements. These supports are either too soft for scale-up applications, or are destroyed by the harsh treatments needed for appropriate sanitation.

The Affi-Prep polymyxin support was developed to meet the requirements of both the research and the process scale laboratory. Because of its unique polymer structure, the Affi-Prep polymyxin support can be sanitized by treatment with 0.1 N sodium hydroxide without affecting its ability to bind endotoxins. The support binds endotoxin molecules with high capacity and selectivity, and displays the high rigidity needed in scale-up applications. Furthermore, Affi-Prep polymyxin beads are macroporous, to allow efficient surface area utilization and high throughput.

FEATURES AND APPLICATIONS

Sanitation with 0.1 N Sodium Hydroxide

Treatment with 0.1 N sodium hydroxide is the standard procedure for sanitizing chromatographic materials used in the production of injectable drugs. As shown in Table 1, the Affi-Prep polymyxin support can be sanitized prior to use by treatment with 0.1 N sodium hydroxide. In this experiment, Affi-Prep polymyxin columns were treated with sodium hydroxide concentrations varying from 0.1 N to 1 N. The results show that the Affi-Prep polymyxin matrix was not affected by any of these treatments, as it retained its ability to bind 99.9% of the endotoxin present in the sample. The Affi-Prep polymyxin support is also stable to 70% ethanol, 100% isopropanol, 1% SDS, and 1% DOC. The recommended regeneration procedure uses 0.1 N NaOH where contact time is kept to a minimum.

Table 1. Endotoxin Binding Activity After Treatment with Various Concentrations of NaOH

NaOH Wash	Total E.U. Applied	E.U. Unbound	% E.U. Removed
0		1.14	99.9
0.1 N		0.84	99.9
0.2 N	13,500	1.35	99.9
0.5 N		1.35	99.9
1.0 N		1.80	99.9

Disposable Poly-Prep™ columns were filled with 0.5 ml of the Affi-Prep polymyxin support and washed with short treatments of varying concentrations of NaOH, followed by washing with endotoxin-free water, and finally 10 mM phosphate, pH 6.0, 100 mM NaCl. Endotoxin (13,500 total E.U.) in the above buffer was then applied and the endotoxin levels in the resulting eluates were determined using the LAL assay.

High Mechanical Strength

The process scale production requirements of the biotechnology industry are best fulfilled by high performance, high throughput chromatographic materials. The Affi-Prep polymyxin support is macroporous (1,000 Å pore size), non-compressible to 1,000 psi, and allows linear flow rates up to 2,000 cm/hr. These characteristics allow practically unlimited scale-up capability and maximize time savings. The ability to perform the regeneration and equilibration steps at very fast flow rates can result in significant reduction of chromatographic turn-around time.

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High Binding Capacity

The theoretical binding capacity of the Affi-Prep polymyxin support is >5 mg of endotoxin per ml of matrix, as measured under equilibrium (batch) conditions with an excess of endotoxin present in 10 mM phosphate buffer, pH 6.0, containing 100 mM NaCl. The high binding capacity of the Affi-Prep polymyxin matrix is a result of both its macroporosity (1,000 Å pore size) and its high polymyxin content (2–4 mg/ml). The large pores allow all endotoxin molecules, including those bound to proteins or other large molecules, to penetrate the beads and interact with a large number of polymyxin molecules inside and outside the beads. Because endotoxins have the ability to bind to proteins and other biologically active molecules, the removal of endotoxins can be extremely difficult. The Affi-Prep polymyxin support binds more than 99.9% of endotoxins, even in the presence of up to 10 mg/ml concentrations of BSA, IgG, or 10% fetal bovine serum, as illustrated in Table 2.

Table 2. Endotoxin Removal in the Presence of Protein

Protein	Concentration	E.U. Applied	E.U. Unbound	% E.U. Removed
Control	No Protein	6,700	2.4	99.9
BSA	0.1 mg/ml	6,700	2	99.9
	1.0 mg/ml		2.2	99.9
	10 mg/ml		1.7	99.9
IgG	0.1 mg/ml	6,700	2.4	99.9
	1.0 mg/ml		4	99.9
	10 mg/ml		7	99.9
FCS	1.0%	6,700	3.5	99.9
	10.0%		6.3	99.9

Aliquots of 70 µl of the Affi-Prep polymyxin matrix were incubated at 20° C for 16 hours in 1.0 ml PBS (10 mM phosphate buffer, pH 7.2, 150 mM NaCl) containing 6,700 E.U. of *E. coli* (055:B5) and the indicated concentrations of protein. The supernatants were assayed for unbound endotoxin by the LAL assay.

High Ligand Stability

The Affi-Prep polymyxin support consists of USP Grade polymyxin B coupled to the Affi-Prep matrix. Any leakage of the polymyxin from the matrix may be an important concern, especially when the matrix is used in pharmaceutical applications. In overnight batch experiments at room temperature, the resin was washed with 0.1 M glycine, pH 10, and no leakage of polymyxin was detected by HPLC methods. This corresponds to a leakage rate of less than 20 ng/ml/hr, or to less than 10 ppm/hr of polymyxin with respect to the polymyxin on the resin. Using a more sensitive biological assay, 0.5–2.0 ppm/hr leakage of polymyxin from the resin was detected in eluates of PBS which had been incubated overnight at 37° C with the resin. The drug quality of the polymyxin used for the Affi-Prep polymyxin support, together with its high ligand stability, should make the Affi-Prep polymyxin support acceptable for most pharmaceutical applications.

Broad Specificity

The Affi-Prep polymyxin support has a broad specificity for binding endotoxin molecules from a number of different strains of gram negative bacteria. Endotoxins derived from these strains are commonly found as contaminants in biological solutions. As shown in Table 3, the Affi-Prep polymyxin support was effective in binding endotoxins from four different *E. coli* strains, as well as *Salmonella abortus*, *Salmonella minnesota*, and *Serratia marcescens*. Thus, the Affi-Prep polymyxin support is effective for the removal of endotoxin molecules from different origins.

Table 3. Removal of Endotoxins Originating from Different Bacterial Strains

Bacterial Strain	E.U. Applied	E.U. Unbound	% E.U. Removed
E. coli 055:B5	3,800	10	99.7
E. coli 0111:B4	2,100	3	99.9
E. coli 0127:B8	5,300	4	99.9
E. coli 0128:B12	2,800	9	99.7
Salmonella abortus	4,000	5	99.9
Salmonella minnesota	1,600	17	98.9
Serratia marcescens	3,000	10	99.7

In this experiment, 70 µl of the Affi-Prep polymyxin matrix was mixed for 16 hours at 20° C in 1.0 ml 10 mM phosphate buffer, pH 7.0, with approximately 1 µg of lipopolysaccharide from the indicated bacterial strains. The supernatants were assayed for unbound endotoxin by the LAL assay.

Product Performance

Packing material	USP Grade polymyxin B coupled to a polymeric matrix
Theoretical endotoxin binding capacity	>5 mg endotoxin/ml Affi-Prep polymyxin matrix
Pressure limit	1,000 psi maximum <300 psi recommended
Regeneration	Short treatments with 0.1 N NaOH
Shipping buffer	Pyrogen free buffer: 0.05 M HEPES, 1 mM EDTA, pH 7.5, containing 0.05% sodium azide
Product formats	25 ml bottle Bulk packaging (direct inquiries to Bio-Rad's Technical Service Department)

References

1. Morrison, D. C. and Jacobs, D. M., *Immunochemistry*, **13,** 813-818 (1976).
2. Issekutz, A., *J. Immun. Methods*, **61,** 275-281 (1983).
3. Talmadge, K. W. and Siebert, C. J., *J. of Chrom.*, **476,** 175 (1989).

Ordering Information

Catalog Number	Product Description
156-0010	**Affi-Prep Polymixin Affinity Support,** 25 ml

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