Affi-Prep® Polymyxin Matrix, 25 ml

Catalog Number 156-0010

Introduction

The Affi-Prep polymyxin support is intended for use in low, medium, or high pressure chromatographic applications. The support consists of polymeric macroporous (1,000 Å) 45 µm beads with USP Grade polymyxin B covalently attached. The main application of this product is the removal of endotoxin molecules from biological and aqueous solutions by affinity chromatography. For best performance, this product should be used in dust-free GMP-grade facilities, all buffers and solutions should be prepared with pyrogen-free water, and, after use, the matrix should be sanitized before use again by treatment with 0.1 N NaOH. Contact time with 0.1 N NaOH should be minimized. Chromatography can be performed at room temperature or at 4° C. If you have any questions concerning this product, contact your local Bio-Rad technical representative or call 1-800-4BIORAD and request bulletin 1429.

General Protocol

1. Pack a suitable chromatography column with the desired volume of the Affi-Prep polymyxin support.

2. Render the column pyrogen-free by treating with 3 column volumes of 0.1 N NaOH. Contact time with 0.1 N NaOH should be minimized. Due to the particle size distribution of the Affi-Prep polymyxin matrix, the flow characteristics under hydrostatic pressure (gravity) can be poor. The matrix requires a pumping system (e.g. peristaltic, medium pressure, HPLC) to be employed. The size distribution and pressure rating of the matrix requires the use of chromatographic columns with a bed support of 20 µm or less.

3. Wash column with 10-15 bed volumes of pyrogen-free water.

4. Equilibrate column with a suitable pyrogen-free buffer (for example: PBS or 10 mM phosphate buffer, 100 mM NaCI, pH 6. Highest binding capacities have been observed when buffers used are 10-50 mM and pH is 6-7. High salt (>0.2 M) can decrease the endotoxin binding under some conditions). Eluate should be neutral and pyrogen-free before starting chromatography.

5. Perform chromatography. Due to the kinetics of the endotoxin/matrix interaction, the speed of sample application and wash depends on the state in which the endotoxins are found in the sample. Free endotoxins will bind relatively fast to the matrix, while solutions containing protein-bound endotoxin will require slower flow rates and/or recycling of the sample over the column several times. Samples containing tightly bound endotoxin contamination may also be incubated overnight at 4° C with the Affi-Prep polymyxin support in a batch mode with gentle shaking.

6. Regenerate column by repeating steps 2 and 3. Equilibration and regeneration steps can be performed at faster flow rates (1,000 cm/hr) than those required for endotoxin binding.

7. Store column in 0.05 M HEPES, pH 7.5, containing 0.05% sodium azide.

Product Performance

Affi-Prep polymyxin matrix	Supplied as a 50% suspension
Theoretical endotoxin binding capacity	>5 mg endotoxin/ml Affi-Prep polymyxin support
pH range	Very stable between pH 2-12; pH 5-8 recommended
Chemical stability	Stable to treatment with 0.1 N NaOH, 70% ethanol, 100% isopropanol, 1% SDS, and 1% DOC
Flow rate	2,000 cm/h maximum; actual flow rates depend upon kinetics of the polymyxin-endotoxin binding reaction
Shipping buffer	0.05 M HEPES buffer, pH 7.5, containing 1 mM EDTA and 0.05% sodium azide

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