Affi-Prep® Protein A Matrix

Instruction Manual

Catalog Numbers
156-0005
156-0006

BIO-RAD

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LIT-230 Rev B

Table of Contents

Section 1
Introduction

Affi-Prep protein A gel consists of highly purified protein A covalently coupled to a unique macroporous polymer matrix. This support is intended for use in medium to high pressure chromatographic applications. All specifications have been developed using the Affi-Prep protein A MAPS® II buffers (catalog number 156-6164).

The usefulness of protein A purification for murine monoclonal antibodies has been limited because, with published methods, most IgG_1 retention represents a significant purification problem. The MAPS buffer system for protein A affinity chromatography was developed to optimize the binding and recovery of many immunoglobulins, especially mouse monoclonal antibodies. The MAPS buffer system has been shown to increase protein A's capacity for IgGs and IgMs from many different species. Approximately 50% of all IgMs will bind to protein A when the MAPS buffer system is used. This buffer system is recommended for use with all protein A affinity supports.

Sample Preparation

Proper adjustment of the pH and ionic streng h of the sample is critical for optimal binding. For best results, the sample pH should be adjusted to 9.0, and the ionic strength of the sample should approach that of the MAPS binding buffer. This can be achieved by sample dilution, dialysis, or buffer exchange using the Econo-Pac® 10DG desalting columns or Bio-Gel® P-6DG gel filtration gel.

- Ascites fluid should be diluted 1:2 with binding buffer. Higher concentrations of binding buffer can enchance the binding of low affinity antibodies.
- Tissue culture supernatant should be concentrated to approximately 5 mg immunoglobulin per ml, and then diluted 1:2 with binding buffer. For large volume samples where further dilution is not desired, we recommend adding the dry binding buffer salts directly to the sample instead of diluting the sample with prepared buffer.
- All samples should be filtered through a 0.45 or 0.8 µm filter before loading onto the column.

Purification Protocol

1. Pack a suitable chromatography column with the desired volume of the Affi-Prep protein A support.
2. Equilibrate the column with 5-10 bed volumes of Affi-Prep MAPS II binding buffer. After equilibration, the pH of the column effluent should be equal to the pH of the binding buffer (pH 9.0).
3. Apply the prepared sample to the column.
4. Wash the column with 10-15 bed volumes of binding buffer to remove all of the unbound contaminating components.
5. Elute the immunoglobulin with 5 bed volumes of Affi-Prep MAPS II elution buffer. Elute with and additional 10 bed volumes of elution buffer to insure total removal of immunoglobulin. Neutralize the eluted sample immediately after elution with 1 M Tris-HC1. (Prolonged exposure of the purified immunoglobulin fraction to acid pH should be avoided.)
6. Regenerate the Affi-Prep protein A column with 50% methanol after every use. The column can be washed with 0.1 N NaOH every 5-10 runs for a more

stringent column wash. This NaOH wash should only be used after the regular methanol regeneration step. For complete sanitation (i.e. removal of endotoxins and DNA) the support can be washed with 1.0 N NaOH. This is an acceptable method of sanitation for FDA purposes.

7. If the column will be re-used right away, re-equilibrate the column with **at least** 5 column volumes of MAPS binding buffer. If the column is to be stored, equilibrate the column with a mild neutral buffer such as 0.05 M sodium phosphate, pH 7.5, containing 0.02-0.05% sodium azide.

Section 2
Production Information

Monoclonal antibody loading capacities	Mouse IgG_1 = 8-10 mg/ml IgG_{2a} = 13-15 mg/ml IgG_{2b} = 13-15 mg/ml IgG_3 = 8-10 mg/ml
Polyclonal antibody loading capacities	Human IgG = 16-23 mg/ml Rat, sheep, bovine, equine, goat, rabbit, dog, and porcine IgG = 9-16 mg/ml IgM = 5-7 mg/ml*
Flow rate	2,000 cm/hr maximum; 150 cm/hr recommended. We recommend the use of the Bio-Rex® MP chromatography columns for medium pressure Affi-Prep protein A applications.
Pressure limit	1,000 psi
Chemical stability	pH 2-14, 1 N NaOH, 8 M urea, 50% methanol, 6 M guanidine-HC1
Shipping buffer	0.05 M sodium phosphate, pH 7.5, containing 0.05% sodium azide
Shelf life	1 year at 4 °C

*Approximately 50% of all IgM's will bind to protein A.

Section 3
Ordering Information

Catalog Number	Product Description
156-0006	**Affi-Prep Protein A Support,** 5 ml
156-0005	**Affi-Prep Protein A Support,** 25 ml
153-6164	**Affi-Prep Protein A MAPS Buffers,** (contains 1.5 L binding and 1.1 L elution buffers)
153-6161	**Affi-Prep Protein A MAPS II Binding Buffer,** 5 liters (contains enough solids to make 5 liters of binding buffer)
153-6162	**Affi-Prep Protein A MAPS II Elution Buffer,** 5 liters (contains enough solids to make 5 liters of elution buffer)

Affi-Prep Protein A Prepacked HPLC Cartridges and Columns

153-6165	**Affi-Prep Protein A MAPS Kit,** contains 1 MAPS II buffers (153-6164), 1 Affi-Prep protein A MAPS analytical cartridge (125-0460), and 1 Standard Cartridge Holder (125-0131)
125-0460	**Affi-Prep Protein A MAPS Analytical Cartridge,** 30 x 4.6 mm
125-0131	**Standard Cartridge Holder,** for 30 x 4.6 mm cartridges

Catalog Number	Product Description
125-0461	**Affi-Prep Protein A MAPS Preparative Cartridge,** 15 x 25 mm
155-0130	**MAPS Preparative Cartridge Holder,** for 15 x 25 mm cartridges
155-0001	**Affi-Prep Protein A MAPS Preparative Column,** 100 x 25 mm
155-0005	**Affi-Prep Guard Cartridge,** 15 x 25 mm, for all 25 mm ID columns and cartridges