Affi-Prep® Protein A MAPS® II Kit

Instruction Manual

BIO-RAD

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Section 1
Introduction

Protein A chromatography is one of the most reliable and specific methods for immunoglobulin purification. The Affi-Prep protein A support is a macroporous affinity chromatography matrix developed to take advantage of the specificity of protein A for medium to high pressure chromatographic applications. This product is particularly useful for the analytical and preparative requirements of the biotechnology industry, where there is a need for scale-up purification of monoclonal and polyclonal antibodies. The Affi-Prep protein A matrix allows high linear flow rates and exhibits excellent mechanical strength. These key features allow the direct transfer of experimental conditions from the initial analytical methods development stage to the final production scale purification of product. When the Affi-Prep protein A matrix is used in conjunction with the optimized Affi-Prep protein A MAPS II buffers, high efficiency binding of a wide range of immunoglobulins, including all isotypes of murine IgG, is achieved. Advantages of the Affi-Prep protein A support include:

- Binds a wide range of monoclonal and polyclonal antibodies, including murine IgG_1
- Macroporous polymeric support is non-compressible under normal operating conditions
- Linear flow rates up to 2,000 cm/hr are achievable
- High flow rates at all column diameters for easy scale-up
- Compatible with most commonly used chromatographic buffers
- Stable pH range from 2–10
- Multiple chromatographic cycles without loss of capacity
- Ligand leakage is extremely low
- Easy to use

1.1 Principle

Protein A, from *Staphylococcus aureus*, has the property of binding with high specificity to the Fc region of immunoglobulins from most mammalian species.[1] When coupled to a suitable hydrophilic support, protein A can be used to purify IgG, to selectively remove IgG prior to analysis of other immunoglobulin classes, or to adsorb immune complexes to purify antigens.[2]

The affinity of IgG for protein A is not the same for all species or isotypes. For this reason MAPS II buffers have been developed to optimize the binding and recovery of many immunoglobulins, including murine IgG_1.

1.2 Comparison of IgG Recoveries

Protein A preparations have been used extensively to purify both polyclonal and monoclonal IgG and IgG subclasses from a variety of mammalian species.[2] Currently, protein A affinity chromatography is being widely used to purify monoclonal antibodies from murine ascites fluid or culture medium supernatants.[3] However, the usefulness of protein A purification in this application has been limited because, using published methods, most IgG_1 immunoglobulins have low affinity for protein A. This results in poor IgG_1 retention on protein A affinity supports.[4-7] Since many murine IgG monoclonal antibodies belong to the IgG_1 subclass, poor IgG_1 retention represents a significant problem in their purification.

Figure 1 shows the results achieved with the Affi-Prep protein A MAPS method, using MAPS II buffers, for the purification of several murine monoclonal antibodies including IgG_1, IgG_{2a}, and IgG_{2b} subclasses.

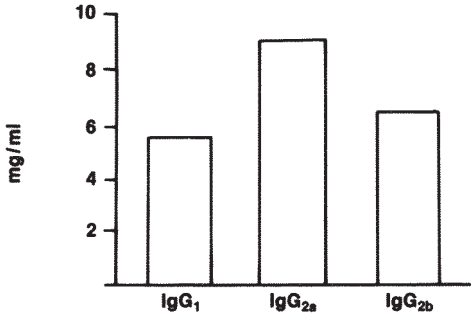

Fig. 1. Binding capacities for murine IgG subclasses. Mouse ascites fluids, containing equivalent amounts (5 mg) of either mouse IgG_1, IgG_{2a}, or IgG_{2b} monoclonal antibody, were purified with 0.5 ml columns of Affi-Prep protein A matrix. Chromatography was performed with the Affi-Prep protein A MAPS II buffers.

1.3 Kit Components

The Affi-Prep protein A MAPS II kit contains enough reagents to perform over 100 analytical cartridge runs, for purification of up to 500 mg of murine IgG.

Affi-Prep protein A: One 30 x 4.6 mm Affi-Prep protein A analytical cartridge (0.5 ml bed volume; catalog number 125-0460) in 0.1 M HEPES, 1 mM EDTA, pH 7.5, containing 0.05% sodium azide.

Cartridge holder: One Standard Cartridge Holder (catalog number 125-0131).

Binding buffer: One bottle (471 g) of buffer solids. Reconstituted final volume = 1,500 ml.

Elution buffer: One bottle (25 g) of buffer solids. Reconstituted final volume = 1,100 ml.

1.4 Additional Items Required

pH meter: A pH meter is required to check the pH of binding and elution buffers after reconstitution.

Mixer: Standard laboratory magnetic stirrer and stir-bar for buffer mixing.

Balance: Standard laboratory scale for weighing of buffer solids.

Section 2
Instructions for Use

2.1 Buffer Preparation

Binding Buffer Preparation

The binding buffer is supplied as a premixed, preweighed solid. Dissolve 31.4 gm binding buffer solids to a final volume of 100 ml using distilled, deionized water. Use the full 471 gm for 1,500 ml. Stir for 10 minutes, or until fully dissolved. Filter through a 0.22 µm membrane filter and check the pH. The pH should be 9.0 ± 0.2. Store buffer at 4 °C. If desired, sodium azide may be added to 0.05% (w/v).

Elution Buffer Preparation

The elution buffer is supplied as a preweighed, premixed solid. Reconstitution and filtration are required prior to use. These salts are hydroscopic. Any material in clumps should be broken up before weighing the solids. Dissolve 2.2 g to a final volume of 100 ml using distilled, deionized water. Use the full 25 g for 1,100 ml. Stir for 10 minutes, or until fully dissolved. Filter through a 0.22 µm filter and check the pH. The pH should be 3.0 ± 0.2. Buffer should be stored at 4 °C.

2.2 Sample Preparation

Proper adjustment of the pH and ionic strength 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, Econo-Pac P6 cartridges, or Bio-Gel® P-6DG gel filtration gel.

- Ascites fluid should be diluted 1:2 with binding buffer. Higher concentrations of binding buffer can enhance the binding of low affinity antibodies.
- Tissue culture supernatant may 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 cartridge.

2.3 Standard Mouse IgG Purification Procedure

1. Place the Affi-Prep protein A analytical cartridge into the Standard Cartridge Holder, making sure that the writing on both are aligned in the same direction. Tighten the end nuts of the holder.
2. Place this assembly in-line in the HPLC or medium pressure system.

   Note: Direction of buffer flow is in the same direction as the lettering on both the analytical cartridge and holder. The inlet side is on the left as you hold the cartridge assembly and read the Bio-Rad logo.
3. Equilibrate the Affi-Prep protein A analytical cartridge with Affi-Prep MAPS II binding buffer for 15 minutes at 0.5 ml/minute (15 column volumes).
4. Dilute the IgG containing sample 1:1 or 1:2 with binding buffer (i.e., 1 volume sample plus 1 or 2 volumes buffer). Filter through a 0.45 µm or 0.8 µm membrane just prior to sample loading. Inject a sample containing up to 2.5 mg of IgG onto the cartridge.

Note: For many murine IgGs the capacity is as high as 10 mg antibody/ml Affi-Prep protein A or 5 mg antibody/Affi-Prep protein A analytical cartridge.

5. Continue flow at 0.5 ml/minute until the unbound fraction of contaminating proteins is completely removed from the matrix (typically within 10 column volumes) and the UV-absorbance trace returns to baseline.
6. Change to Affi-Prep MAPS II elution buffer and return flow to 0.5 ml/minute. The IgG will typically elute at, or slightly behind, the elution buffer/binding buffer interface.

   Note: For many samples, a less acidic release buffer is sufficient. If a particular IgG is acid sensitive, alternative elution buffers should be tested.
7. Store the Affi-Prep cartridge in a mild, neutral storage buffer. Recommended is 0.1 M HEPES, 1 mM EDTA, pH 7.5, containing 0.02–0.05% sodium azide.
8. Flush buffer pump(s) with storage buffer or water prior to system shutdown. This greatly reduces the possibility of damage to non-resistant fluid paths, since MAPS II buffers contain halide salts that can form deposits on standing.

Section 3
Answers to Common Questions

1. Regeneration of the cartridge:

Affi-Prep protein A matrix need not be regenerated. Affi-Prep protein A matrix has been recycled reproducibly up to 550 times without regeneration.

If regeneration is desired, regenerate the Affi-Prep protein A cartridge with 50% methanol after every use. The cartridge can be washed with 0.1 N NaOH every 5-10 runs for a more stringent 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 cartridge can be washed with 1.0 N NaOH.

If the cartridge will be re-used right away, re-equilibrate the cartridge with at least 5 cartridge volumes of MAPS binding buffer. If the cartridge is to be stored, equilibrate the cartridge with a mild neutral buffer such as 50 mM sodium phosphate, pH 7.5, containing 0.02–0.05% sodium azide.

2. Sensitivity of antibodies to low pH:

Some antibodies can be inactivated by low pH. Inactivation may be reduced by collecting the eluted immunoglobulin fraction into a concentrated, high pH buffer, or the fraction can be dialyzed against a buffer of choice. In cases of extreme sensitivity, many immunoglobulins can be eluted at pH 4-6 by raising the pH of the elution buffer with 1.0 N NaOH.

3. Flow rate of the Affi-Prep protein A analytical cartridge:

Maximum flow rate is 2.0 ml/minute; 0.5 ml/minute is recommended for maximum binding efficiency for viscous samples (e.g., ascites fluid).

4. Purification of IgG from species other than mouse:

When the specially optimized Affi-Prep protein A MAPS II buffers are used in conjunction with the Affi-Prep protein A matrix, a broad range of monoclonal and polyclonal antibodies can be purified. Representative capacities for porcine, canine, bovine, murine, and rabbit are in the range of 9-16 mg polyclonal IgG per ml matrix.

5. Shelf life of the Affi-Prep protein A MAPS II kit:

The shelf life of Affi-Prep protein A matrix is at least 1 year at 4 °C. The Affi-Prep MAPS II buffer solids are stable for at least 1 year when stored at room temperature. Store the reconstituted buffers at 4 °C for up to 2 weeks.

Section 4
References

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Section 5
Product Information

Catalog Number	Product Description
156-0006	**Affi-Prep Protein A Matrix**, 5 ml
156-0005	**Affi-Prep Protein A Matrix**, 25 ml
125-0460	**Affi-Prep Protein A Maps Analytical Cartridge**, 30 x 4.6 mm, requires 125-0131 holder
125-0131	**Standard Cartridge Holder**, for 30 x 4.6 mm cartridges
125-0461	**Affi-Prep Protein A MAPS Preparative Cartridge**, 15 x 25 mm, requires 155-0130
155-0130	**Preparative Cartridge Holder**, for 15 x 25 mm cartridges
153-6164	**Affi-Prep Protein A MAPS II Buffers**, includes solids to make 1.5 liters binding buffer and 1.1 liters elution buffer
153-6165	**Affi-Prep Protein A MAPS II Kit**, includes 125-0460, 125-0131, 153-6164, and instructions
153-6161	**Protein A MAPS II Binding Buffer**, 5 liters
153-6162	**Protein A MAPS II Elution Buffer**, 5 liters

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