Affi-Gel® Protein A MAPS® II Kit

Instruction Manual

Catalog Number 153-6159

For Technical Service
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Introduction

The Affi-Gel protein A MAPS II (Monoclonal Antibody Purification System) kit provides a dramatic improvement in protein A-agarose methods for purification of mouse IgG_1 from ascites fluid. When Affi-Gel protein A agarose is combined with specially optimized MAPS II binding, elution, and regeneration buffers, protein A capacity for mouse IgG_1 from ascites fluid is 6-8 mg/ml gel. This capacity is 8-10 times higher than that obtained with published methods.[1,2]

Affi-Gel protein A agarose is purified protein A coupled to crosslinked agarose beads, with an exclusion limit of greater than 10 M daltons, via chemically stable amide bonds. This coupling chemistry, plus the stability of native protein A, results in excellent resistance to denaturing agents such as urea, chaotropic salts such as guanidine hydrochloride or potassium thiocyanate, and acid and base (pH 2-11). Affi-Gel protein A agarose contains approximately 2 mg of protein A/ml gel.

Principle

Protein A, from *Staphylococcus aureus*, has the property of binding with high specificity to the Fc region of Ig from most mammalian species.[3] When coupled to agarose beads, protein A can be used to purify IgG, to selectively remove IgG prior to analysis of other Ig classes, or to absorb immune complexes to purify antigens.[2]

The affinity of IgG for protein A is not the same for all species. For this reason MAPS II buffers have been developed to optimize binding and recovery of mouse IgG_1.

Comparison of IgG Recoveries

Protein A-agarose preparations have been used extensively to purify IgG and IgG subclasses from a variety of mammalian species.[4] Currently, protain A-agarose is being widely used to purify monoclonal antibodies from mouse ascites fluid or culture medium supernatents. However, the usefulness of protein A-agarose in this

application has been limited because most mouse IgG_1 immunoglobulins have low affinity for protein A. This results in poor IgG_1 retention on protein A-agarose columns.[1,5-7] Since most mouse IgG monoclonal antibodies belong to the IgG_1 subclass, poor IgG_1 retention represents a significant purification problem.

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

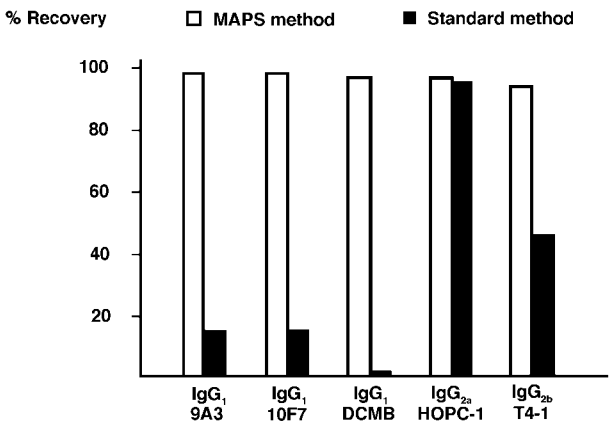

Fig. 1. Comparison of IgG_1 recoveries using Affi-Gel protein A MAPS method and standard method.

Mouse ascites fluid containing equivalent amounts (5 mg) of either mouse IgG_1, IgG_{2a}, or IgG_{2b} monoclonal antibodies were chromatographed in 1 ml columns of Affi-Gel protein A agarose. Chromatography was performed with the Affi-Gel protein A MAPS kit. It is evident that almost quantitative recovery was achieved in all 3 cases in which IgG_1-containing mouse ascites was chromatographed using the MAPS kit. Improved results were also observed with IgG_{2b} antibodies.

Kit Components

The Affi-Gel protein A MAPS II kit contains a sufficient quantity of reagents to purify approximately 500 mg of mouse IgG_1.

Affi-Gel protein A agarose	One bottle containing 5 ml of gel suspended in 0.05 M sodium phosphate, pH 7.5, with 0.05% NaN_3.
Binding buffer	One bottle (471 g) of buffer solids. Reconstitution volume = 1,500 ml.
Elution buffer	One bottle (25 g) of buffer solids. Reconstitution volume = 1,100 ml.
Regeneration buffer	One bottle containing 400 ml. **Caution:** Contains methanol. **Do not mouth pipette.**
Column	1 x 10 cm Econo-Column® chromatography column.

Additional Items Required

Test tubes	General purpose tubes for fraction collection are recommended.
pH meter	A pH meter is required to check the pH of the binding and elution buffers after reconstitution.
Mixer	Standard laboratory magnetic stirrer and bar for buffer mixing.
Balance	Standard laboratory scale for weighing out buffer solids.
Filter	0.22 micron filter for buffer preparation.

Instructions for Use

Binding Buffer Preparation

The binding buffer is supplied as a premixed, preweighed solid. Reconstitution and filtration are required prior to use. Dissolve 31.4 g binding buffer solids per 100 ml distilled, deionized water. (Use the full

471 g for 1,500 ml.) Stir for 10 minutes. Filter through a 0.22 µ nylon filter and check the pH. The pH should be 9.0 ± 0.2. If the pH is not in this range, adjust the pH with 10 N NaOH or 6 N HCl. Store buffer solids at room temperature. Store reconstituted 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 hygroscopic. Any material in clumps should be broken up before you weigh any solids. Dissolve 2.2 g per 100 ml distilled deionized water (use the full 25 g for 1,100 ml). Stir for 10 minutes. Filter through a 0.22 µ filter and check the pH. The pH should be 3.0 ± 0.2. If the pH is not in this range, adjust the pH with 10 N NaOH or 6 N HCl. Store buffer solids at room temperature. Reconstituted buffer should be stored at 4 °C, and if desired, Thimerosal bacteriostat may be added to a final concentration of one part per ten thousand. The use of sodium azide in low pH buffers is not recommended.

Sample Preparation

Ascites samples should be diluted one to one with binding buffer. If your sample is tissue culture supernatant, it should be concentrated to approximately 5 mg immunoglobulin/ml and then diluted one to one with binding buffer.

Standard Mouse IgG_1 Purification Procedure

1. Pack a 1 x 10 cm Econo-Column chromatography column (supplied) with the desired volume of Affi-Gel protein A agarose. (One ml of Affi-Gel protein A agarose will bind 6-8 mg/ml mouse IgG_1.)
2. Equilibrate the column with 5 bed volumes of binding buffer. After equilibration, the pH of the column effluent shold be equal to the pH of the binding buffer (pH 9.0).

3. Apply diluted sample to the column.
4. Wash the column with 15 bed volumes of binding buffer.
5. Elute the IgG with 5 bed volumes of elution buffer. Elute column with an additional 10 volumes of buffer to insure total removal of IgG. Prolonged exposure to acid pH should be avoided. Neutralize eluate immediately after elution. For example, for a 1 ml column, collect eluate in a tube containing 1.6 ml of 1 M Tris HCl, pH 9. Sample pH under these conditions is 6 to 8. Alternatively, eluate can be dialyzed against the buffer of choice, or desalted over Bio-Gel® P6DG desalting gel. See bulletin 2068 for desalting protocols.
6. Wash the column with 5 bed volumes of regeneration buffer.
7. Store the PBS containing 0.05% sodium azide.

Note: For all sample sizes, short columns (10-15 cm or shorter) are recommended. The better linear flow rates obtained in short columns facilitate the washing and regeneration steps.

Answers to Common Questions

1. Regeneration of column:

   Affi-Gel protein A agarose can be regenerated 10-12 times with the regeneration buffer supplied. We strongly recommend regenerating the column each time it is used to increase the lifetime of the gel to insure consistent separations from run to run. Cross-over contamination of IgG from application to application will also be eliminated.

2. Sensitivity of antibodies to low pH:

   There are a few antibodies that are inactivated by low pH. Inactivation can be avoided by collecting the fraction eluted into a concentrated neutral buffer. In cases of extreme sensitivity, many immunoglobulins can be eluted at pH 4-6 by bringing up the pH of the elution buffer with 10 N NaOH.

3. Flow rate of Affi-Gel protein A agarose:

The flow rate will be greater than or equal to 0.6 ml/min for a 1 ml column with an ID of 1 cm and a 2:1 buffer to gel ratio. A typical sample run will take 1.0 to 1.5 hours for a one ml column.

4. Purification of IgG other than mouse with the Affi-Gel protein A MAPS II kit:

   Rabbit IgG from serum has been purified successfully with the Affi-Gel protein A MAPS II kit (Bio-Radiations No. 53). The protocol in this booklet can be used without modification.

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

   The shelf life of Affi-Gel protein A agarose is 1 year at 4 °C. The unreconstituted buffers are good for at least 1 year when stored at room temperature.

References

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Product Information

Catalog Number	Product Description
153-6153	**Affi-Gel Protein A Agarose**, 5 ml
153-6154	**Affi-Gel Protein A Agarose**, 50 ml
153-6159	**Affi-Gel Protein A MAPS II Kit**, includes 5 ml Affi-Gel protein A, binding, elution, and regeneration buffers, and 1 x 10 cm Econo-Column chromatography column.
153-6160	**Affi-Gel Protein A MAPS II Buffers**, includes binding, elution, and regeneration buffers.
153-6156	**Protein A**, 10 mg
153-6157	**Protein A**, 100 mg
153-6161	**Affi-Gel Protein A MAPS II Binding Buffer**, 5 liters
153-6162	**Affi-Gel Protein A MAPS II Elution Buffer**, 5 liters

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SIG 020996 Printed in USA

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