Affi-Gel® Protein A Agarose for IgG Purification

Protein A, from *Staphylococcus aureus*, has the property of binding with high specificity to the F_C region of Ig, usually IgG from mammalian species.[1] When coupled to agarose beads, protein A can be used to purify IgG, to selectively remove IgG prior to analysis of other Ig isotypes, to absorb immune complexes for antigen purification, or as second antibody in ELISA assays (see Reference 2 for review).

Affi-Gel protein A agarose is purified protein A coupled to crosslinked agarose beads 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 gel contains approximately 2 mg of protein A/ml gel. It has a binding capacity of ≥ 18 mg human IgG/ml gel.

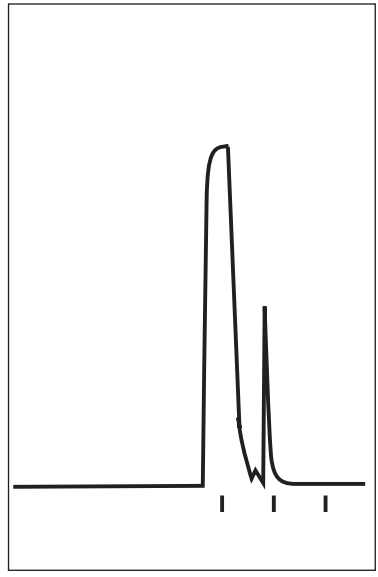

Fig. 1. Affi-Gel protein A chromatography of human serum. 1 ml of human serum was chromatographed in a 1 ml column of Affi-Gel protein A agarose as indicated in instructions for use. Elution of immunoglobulin with pH 3 buffer was started at 16 minutes.

Instructions for Use

For maximum binding capacity, the MAPS® II buffer system is recommended. This system contains carefully optimized binding, washing, and elution buffers which enhance binding of IgG to protein A, and significantly increase the capacity also for murine IgG.

1. Prepare column of Affi-Gel protein A agarose. For example, a 1 ml column of gel is sufficient to bind the IgG from 1 ml human serum (typically 6-16 mg IgG/ml). Poly-Prep® chromatography columns (catalog number 731-1550) are recommended for small samples such as this.

2. Equilibrate the column with 10 bed volumes of binding buffer. For the above example, 10 ml of buffer would be required. A typical binding buffer is 10 mM sodium phosphate, 0.15 M sodium chloride, pH 8.2.
3. Adjust the pH of the sample to equal that of the binding buffer.
4. Apply the sample to the column.
5. Wash the column with 10 bed volumes (10 ml for the example) or until the O.D. at 280 nm of the column eluant approaches zero.
6. Elute the immunoglobulin fraction with 0.1 M sodium citrate, pH 3.0. Generally 2-5 column volumes are required for quantitative removal of Ig.
7. Wash the column with 3 bed volumes of 1.5 M sodium thiocyanate.
8. Wash the column with 3 bed volumes of binding buffer.
9. Store the column at 4 °C. If the column is to be stored for more than a few days make binding buffer 0.05% in sodium azide.

References

1. Kronvall, G. and Williams, R. C., *J. Immunol.*, **103,** 828 (1969).
2. Lindmark, R., Thoren-Tolling, K. and Sjoquist, J., *J. Immunol. Methods*, **62,** 1 (1983).

Ordering 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 of Affi-Gel protein A gel, 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
153-6161	**Protein A MAPS II Binding Buffer,** 5 liters
153-6162	**Protein A MAPS II Elution Buffer,** 5 liters