AP1760 Protein A-agarose

Descriptions: Prepared with recombinant streptococcal Protein A from which the albumin-binding region has been genetically deleted.
Appearance: White suspension
Binding Capacity: >20 mg Human IgG/ml
Form: 50% suspension in 10 mM sodium phosphate containing 0.02% sodium azide.
Extent of labeling: 3 mg/mL
Matrix: Sepharose 4B
Activation: Cyanogen bromide
Specificity: mouse IgG2a, IgG2b, and IgA, rabbit IgG, and human IgG1, IgG2 and IgG4.
Stability: Protein A immobilized on agarose is stable to all commonly used aqueous buffers. The working pH range of the resin is 3-9 (long-term) or 2-10 (short term).
Storage: 2-8°C

Usage:
Protein A binds IgG at neutral to basic pH. The sample matrix can be serum, ascites fluid, or any low ionic strength neutral buffer, such as 20 mM sodium phosphate buffer, pH 7.0. For affinity chromatography, clarify samples that are cloudy or have particulate matter by filtration or by centrifugation.
Load the sample onto the column. When the fluid layer reaches the top of the resin bed, wash with 1-2 column volumes of starting buffer or 20 mM sodium phosphate buffer, pH 7.0. Wash the column with an additional 3-4 column volumes of starting buffer or 20 mM sodium phosphate buffer, pH 7.0. Elute IgG with 1-3 column volumes of 100 mM glycine HCl, pH 2.7. Bring the fractions containing IgG to neutral pH with 1 M tris HCl, pH 9.0 or 0.1 N NaOH. Reequilibrate the resin with a neutral buffer.

IgG purification by immunoprecipitation:
Add an appropriate volume of washed resin to the sample. Mix the sample and resin on a rocking or oscillatory shaker for 1 hr. Collect the resin on a Buchner funnel under gentle vacuum, and wash the resin with ten resin volumes of working buffer or 20 mM sodium phosphate buffer, pH 7.0. Resuspend the resin in 2-3 resin volumes of acidic buffer, such as 100 mM glycine HCl buffer, pH 2.7. Mix on a rocking or oscillatory shaker for 15 minutes to elute the IgG. Collect the eluate by vacuum filtration or by centrifugation. Bring the eluate to neutral pH with 1 M tris HCl, pH 9, or 0.1 N NaOH. If the resin is to be reused, equilibrate it with a neutral buffer.

Cleaning:
The resin does not need to be cleaned after each use. However, if serum or ascites fluid has been run on the resin, it can be sanitized with 70% ethanol.
If the binding capacity of the resin is reduced, it may be restored by removing non-specifically bound proteins. Generally, these proteins will elute in 1-5 M NaCl solution, 100 mM Tris or borate buffer, pH 8.5, containing 0.5 M NaCl, or 100 mM sodium acetate buffer, pH 4.0, containing 0.5 M NaCl. If this does not restore binding capacity, any of the following solutions can be used: 0.1 N HCl; 100 mM glycine NaOH buffer, pH 11; 6 M urea; 8 M guanidine HCl, or 1% SDS. Reequilibrate the resin with 4-5 column volumes of a neutral buffer. If the resin is to be stored, add a bacteriostat to the final buffer wash.

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