ALPHA DIAGNOSTIC INTERNATIONAL

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Product Data Sheet

Recombinant Protein A-Agarose

Cat #. PRTA15-AS-1	**Size**: 1 ml	**Cat #.** PRTA15-AS-5	**Size**: 5 ml
Cat #. PRTA15-AS-25	**Size**: 25 ml	**Cat #.** PRTA15-AS-100	**Size**: 100 ml

Description

Protein A is a cell wall protein deriving from Staphylococcus aureus which exhibits unique binding properties for IgG from a variety of mammalian species and for some IgM and IgA as well. It binds with the Fc region of immunoglobulins through interaction with the heavy chain. It couples to a wide variety of reporter molecules including fluorescent dyes, enzyme markers, biotin, colloidal gold and radioactive iodine without affecting the antibody binding site. Recombinant Protein A was developed to increase the specificity of the molecule for IgG and is widely used both in research and bioprocessing. The recombinant protein A is produced by expressing a modified protein A gene in E.coli. A specific purification process with strict quality control was taken to get the recombinant protein A with the purity of more than 98% , no human IgG affinity step is used during validated fermentation and purification and devoid of bacterial contaminant found normally in native Protein A. (Free of Staphylococcus endotoxins and hemolysin).

Recombinant Protein A from Staphylococcus aureus Cowan I is expressed in Bacillus and purified (>98%, ~45 kDa). It has been purified using a proprietary method without using human IgG-Agarose to avoid contamination with IgG.

Purified Protein-A was coupled to Agarose using a unique immobilization chemistry that retains the maximum IgG-binding capacity and minimum Protein-A leaching (<5 ng rProtein-A/ml).

Binding capacity-
20-40 mg human IgG/ml of Protein A-Agarose.

Form and Storage

Protein-A-Agarose is supplied in PBS, pH 7.4 containing 0.1% azide as preservative (50% v/v suspension). Store at 4oC. Do not freeze and thaw.

General Usage

1. Make a 1:1 suspension of resin in Buffer A. Pour into column. Allow column to flow as it is settling. After it has settled, wash with 20 column volumes (CV) of Buffer A.
2. Apply sample slowly and recycle the eluant 2-3 times if necessary.
3. Wash with 10 CV of buffer A.
4. Elute with 3 CV of Buffer B. Collect fractions. Neutralize the eluate with 0.1 M NaOH. Assay the eluate for IgG. Re-equilibrate the column with 20-30 CV of Buffer A.
5. Store in Buffer A with a preservative at 2-8℃. If solution volume is significantly greater than the resin volume, column method is recommended.

Buffer A: 0.02M NaPO4, 0.15M NaCl, pH 8.0
Buffer B: 0.2M NaPO4, 0.1M Citric Acid, pH 4-7 (depending upon the species IgG).

Regeneration and Cleaning of Protein-A Agarose

A decrease in the binding capacity may be due to steric hindrance by non-specifically bound proteins. It may be possible to clean the resin by washing it with 10-20 volumes of 100 mM Tris or borate buffer, pH 8.5, containing 0.5-2.0 M NaCl, followed by 10-20 volumes of 100 mM acetate buffer, pH 4.0, containing 0.5-2.0 M NaCl. Reequilibrate the resin with 20 volumes of buffer A. Add preservative and store at 2-8℃.

Species IgG Binding Capacity and Elution pH

Species	Subclass	Binding	Elution (pH)
Human	IgG	High	4
	IgG1	High	3.9-4.6
	IgG2	High	4.3-5
	IgG3	----	4
	IgG4	High	3.9-5
Mouse	IgG1	Low	6-7
	IgG2a	High	4.5-5
	IgG2b	High	4.5
	IgG3	High	3.5-4
Rabbit	IgG	High	3
Rat	IgG1	Low **	7
	IgG2a/2b	-----	
	IgG2c	Medium-high	3-4
	IgG1	Low	7
	IgG1	Low	7
	IgG1	Low	7
	IgG1	Low	7
G. Pig	IgG	High	4
Bovine	IgG	Low	
Goat	IgG	**	

**=Capacity may be increased by using alternative buffers: 1 M glycine, 2 M NaCl, pH 9 or 1 M borate, 2 M NaCl, pH 9. With mouse IgG1, use a higher pH (9), and a sodium chloride concentration of 2-3 M. Elute with a gradient to pH 3, 0.15 M NaCl.

For in vitro research use only

Related Material available for ADI

Recombinant Protein-A/G, Coated ELISA plates, Antibodies to Protein A/G,

Protein A and G ELISA kits

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