

Biotin Conjugated Protein A

Basic Information

Catalog Number	BA1025-0.5
Size	0.5 mL
Concentration	1 mg/mL
Host	E. coli
Conjugation	Biotin
Form	Concentrated, Liquid
Tested Application	IHC, ELISA
Contents	0.5 mg of biotin conjugated protein A, Biotin: Ab=10:1, 0.01 M PBS, 50% glycerol.
Specificity	Biotin conjugated antibody is specific for Protein A, human IgG1, IgG2 and IgG4 bind strongly, while IgG3 does not bind. There are also many instances in which monoclonal antibodies do not bind to Protein A, especially the majority of rat immunoglobulins and mouse IgG1.

Suggested Working Concentration

Immunohistochemistry: 5-10 µg/mL

ELISA: 0.05-0.2 µg/mL

Optimal working dilutions must be determined by end users.

Application Notes

This antibody must be used with various kinds of enzymes or fluorescence conjugated avidin (streptavidin) in IHC and ELISA.

Dilute with neutral PBS or TBS.

Storage

At -20° C for one year from date of receipt. Avoid repeated freezing and thawing.

Product Description

Protein A is a 42 kDa surface protein originally found in the cell wall of the bacteria *Staphylococcus aureus*. It is encoded by the *spa* gene and its regulation is controlled by DNA topology, cellular osmolarity, and a two-component system called ArlS-ArlR. It has found use in biochemical research because of its ability to bind immunoglobulins. It is composed of five homologous Ig-binding domains that fold into a three-helix bundle. Each domain is able to bind proteins from many mammalian species, most notably IgGs (Table 1). It binds the heavy chain within the Fc region of most immunoglobulins

and also within the Fab region in the case of the human VH3 family. Through these interactions in serum, where IgG molecules are bound in the wrong orientation (in relation to normal antibody function), the bacteria disrupts opsonization and phagocytosis. Recombinant Protein A is produced in E.coli and essentially functions the same as native Protein A. Protein A may be conjugated with various reporter molecules, including fluorescent dyes (FITC), enzyme markers (peroxidase, b-galactosidase, and alkaline phosphatase), biotin, and colloidal gold without affecting the antibody binding site on the molecule. These conjugates are used to detect immunoglobulins in various immunochemical assays including Western blotting, immunohistochemistry, and ELISA applications.

Table 1
Binding capabilities for immunoglobulin proteins and protein A, G and A/G

Species	Antibody Class	Protein A	Protein G	Protein A/G
Human	Total IgG	S	S	S
	IgG1	S	S	S
	IgG2	S	S	S
	IgG3	W	S	S
	IgG4	S	S	S
	IgM	W	NB	W
	IgA	W	NB	W
	IgD	NB	NB	NB
Mouse	Total IgG	S	S	S
	IgG1	W	M	M
	IgG2a	S	S	S
	IgG2b	S	S	S
	IgG3	S	S	S
	IgM	NB	NB	NB
Rat	Total IgG	W	M	M
	IgG1	W	M	M
	IgG2a	NB	S	S
	IgG2b	NB	W	W
	IgG2c	S	S	S
Cow	Total IgG	W	S	S
	IgG1	W	S	S
	IgG2	S	S	S
Goat	Total IgG	W	S	S
	IgG1	W	S	S
	IgG2	S	S	S
Sheep	Total IgG	W	S	S
	IgG1	W	S	S
	IgG2	S	S	S

Horse	Total IgG	W	S	S
Rabbit	Total IgG	S	S	S
Guinea Pig	Total IgG	S	W	S
Pig	Total IgG	S	W	S
Dog	Total IgG	S	W	S
Cat	Total IgG	S	W	S
Chicken	Total IgY	NB	NB	NB

Binding will only occur if the appropriate kappa light chains are present. The binding affinity only refers to species and subtypes with the correct kappa light chains. Lambda light chains and some kappa light chains will not bind.

Notes: W= weak binding M=medium binding S=strong binding NB=no binding