Table 1. Protein A and G Binding Capacities for Various Species

Species	Immunoglobulin	Protein A Binding	Protein G Binding
Rabbit	IgG	Strong	Strong
Human	IgG	Strong	Strong
	IgG1	Strong	Strong
	IgG2	Strong	Strong
	IgG3	None	Strong
	IgG4	Strong	Strong
	IgA	None	None
	IgM	None	None
Mouse	IgG1	Weak	Strong
	IgG2a	Strong	Strong
	IgG2b	Strong	Strong
	IgG3	Moderate	Strong
Rat	IgG	None	Weak-Strong
Goat	IgG	Weak	Moderate
Sheep	IgG	Weak	Moderate
Chicken	IgG	None	Weak
Guinea Pig	IgG	Strong	Moderate
Hamster	IgG	Weak	Moderate
Horse	IgG	Moderate	Strong
Pig	IgG	Strong	Strong
Bovine	IgG	Moderate	Strong
Dog	IgG	Strong	Strong
Cat	IgG	Strong	None

Related Material available from ADI

Catalog#	Product Description
PRTA11-R-5	Recombinant purified >98% (E. coli) Protein A
PRTA12-A	Anti-Protein-A IgG aff pure
PRTA12-BTN	Anti-Protein-A IgG-biotinylated
PRTA13-A	Anti-Protein-A IgG aff pure
PRTA13-HRP	Anti-Protein-A IgG-HRP conjugate
PRTA15-AS-5	Recombinant Protein A-Agarose, affinity matrix
PRTA55-5P	Protein A-coated ELISA plate
PRTG15-R-1	Recombinant purified (>95%) Protein G
PRTG65-5P	Protein G-coated ELISA plate
800-110-PRA	Protein A Quantitative ELISA kit
800-120-PRG	Protein G Quantitative ELISA kit

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. M- PRTAG75-5P

Protein A/G Coated ELISA Plates

Cat. # PRTAG75-5P

Protein A/G Coated 96-well ELISA Plates

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INTRODUCTION

Proteins A and G specifically bind the Fc regions of immunoglobulins of many mammalian species (see Table 1), with an orientation that allows the Fab binding sites to be freely available for efficient binding to epitope. When coated onto microplates, the Protein A/G can securely capture IgG applied directly or as antigen/antibody complexes.

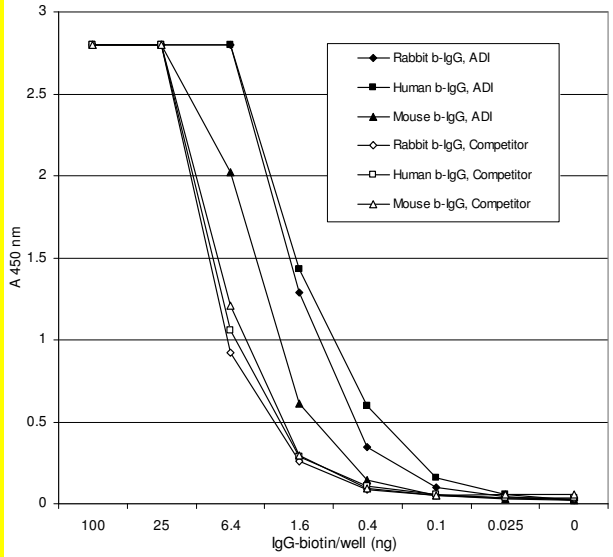
Protein A/G Coated Microplates, Cat# PRTAG75-5P, are coated with genetically-engineered, truncated forms of proteins A and G, designed to reduce non-specific binding to proteins other than the Fc region of IgG. The plates are post-coated (pre-blocked) to further reduce non-specific binding, maintain stable activity, and to provide the convenience of direct use.

Applications for the use of the plates include the following:

- Capture and analysis of antibodies using labeled antigens
- ELISA
- Protein-protein interactions
- Isolation and analysis of fusion proteins or native proteins

Note: When using the plates for a multiple antibody assay, such as a sandwich ELISA, the second Ab can bind to the Protein A/G on the plate to produce falsely positive signals. Therefore, a non-Protein A/G binding Ab, such as from chicken or an $(Fab')_2$, used as detector (e.g., HRP or ALP conjugate) must be used.

Figure 1. Relative Sensitivities of Protein A Plate:
Mammalian IgGs (biotinylated);
Alpha Diagnostics vs Competitor Plate



SPECIFICATIONS

Components
Five (5) individually pouched 96-well plates, configured in 12 removable 8-well strips.

Coating
Recombinant Protein A, from Staphlococcus aureus Cowan I expressed in Bacillus and Recombinant Protein G, from Staphlococcus Group G expressed in E. coli, are coated using 100ul/well. Nominal binding capacity is ~10 pmol IgG/well. The strips are post-coated (blocked)for low non-specific binding and long-term stability.

Storage and Stability
The microplates, if unopened, are stable refrigerated until the expiration date printed on the label. If opened, store in closed pouch with desiccant and use within 2-4 weeks.

APPLICATION

Strategy for Protein Analysis by SDS-PAGE

Materials Required

- Protein A/G Coated Plate, Cat# PRTAG75-5P
- Antibody to specific antigen of interest
- Cell lysate or soluble preparation containing specific antigen of interest

Protocol

1. Mix the antibody with the cell lysate and incubate for >1 hour.

   [Ab binds to Ag]

2. Add 200ul of Ab/lysate mixture to each Protein A/G well and incubate at room temperature for >2 hours, or overnight at 4° C.

   [Ab/Ag complex binds to Protein A]

3. Wash the wells 3 times (250ul) with buffer/detergent (e.g., PBS + 0.05% Tween 20).
4. Add a small volume (e.g., 30ul) of SDS-PAGE Sample Buffer to the well, mix by hand or vortex to wash the sides of the well, and incubate at 95-100° C for 5 minutes.
5. Apply the well contents directly onto an SDS-PAGE gel and run according to manufacturer's instructions.
6. Gels may be stained or analyzed by Western Blot using standard procedures.