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

Quality Control of Protein A or Protein G Coated ELISA plates

ADI has formulated **biotinylated rabbit IgG** that can be used as positive control for the Protein A or Protein G coated plates. **Streptavidin-HRP** conjugate can be used for detection.

Related Material available from ADI

Catalog#	ProdDescription
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 Potein A-Agarose, affinity matrix
PRTG65-5P	Protein A-coated ELISA plate (8 well strips, 96 wells/plate) 5 plates/pack
PRTAG75-5P	Protein A&G-coated ELISA plate (8 well strips, 96 wells/plate) 5 plates/pack
PRTG15-R-1	Recombinant purified (>95%) Protein G
PRTG65-5P	Protein G-coated ELISA plate (8 well strips, 96 wells/plate) 5 plates/pack
20009-BT-1	**Rabbit IgG-Biotinylated**
20365	**Streptavidin-Peroxidase (HRP) conjugate**
Protein A & G ELISA kit	Please call for details

Instruction Manual No. M- PRTG65-5P

Protein A Coated ELISA Plates

Cat. # PRTG65-5P

Protein G Coated 96-well ELISA Plates

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INTRODUCTION

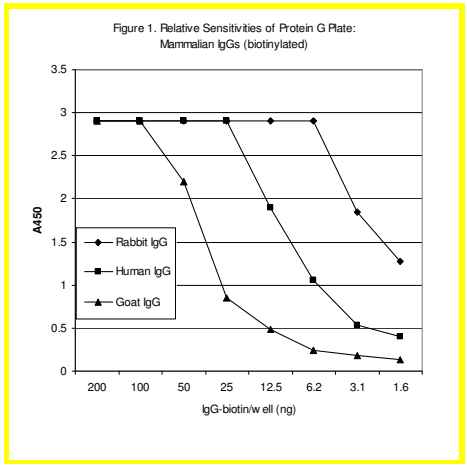
Protein G is a bacterial cell wall protein isolated from group G *Streptococci*. Native Protein G contains two IgG-binding domains and sites for albumin and cell surface binding. The albumin and cell surface binding domains have been eliminated from purified Recombinant Protein G to reduce nonspecific binding and, therefore, can be used to separate IgG from crude samples. Optimal binding occurs at pH 5, although binding is also good at pH 7.0-7.2. Protein G has greater affinity than Protein A for most mammalian IgGs, it may be used for the purification of mammalian IgGs that do not bind well to Protein A. Protein G does not bind to human IgM, IgD and IgA.

Native protein is approx. 65-kda. It contains two albumin binding domains at the N-terminus and 3 IgG binding domains (~55-amino acids each) at the C-terminus. Recombinant protein G does not contain albumin binding domains. Differences in binding characteristics between Protein A and Protein G may be explained by the differing compositions in the IgG-binding sites of each protein, and sequence differences between Protein A and Protein G. Variations in isolation and purifications methods for Protein G may affect IgG binding, partially because there are differing numbers of IgG-binding sites on various sources of Protein G.

Protein G Coated ELISA plates, Cat# PRTG65-5P, are coated with a genetically-engineered, truncated form of protein G from Staphylococcus Group G is expressed in E. Coli and purified (>98%, mol wt ~23 kda but migrate as ~32 kda protein). The gene has been altered to remove the albumin binding domains. This does not affect the protein biding to 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 on the plate to produce falsely positive signals. Therefore, a non-Protein A binding Ab, such as from chicken or an (Fab')2, used as detector (e.g., HRP or ALP conjugate) must be used.



SPECIFICATIONS

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

Coating
Recombinant Protein G is coated using 100ul/well. Nominal binding capacity is ~5 pmol IgG/well. The strips are post-coated (blocked)for low non-specific binding and long-term stability.

Sensitivity
Biotinylated IgG was detected at concentrations significantly above background in an ELISA format using streptavidin-HRP as detector and TMB as substrate (see Figure 1), as follows:

Rabbit IgG – 0.7 ng/well
Human IgG – 2.1 ng/well
Goat IgG – 7.1 ng/well

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.

APPLICATIONS

Strategy for Protein Analysis by SDS-PAGE

Materials Required

- Protein G Coated Plate, Cat# PRTG65-5P
- 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 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.

Gels may be stained or analyzed by Western Blot using standard procedures.