PROTEIN A/G COATED SURFACE

The Biomat product is a 96 well coated microplate with recombinant Protein A/G and a protein to block non-specific binding sites and to maintain stable activity.
Protein A/G includes four Fc binding domains from Protein A and two from Protein G making it a more versatile tool.
Protein A/G specifically binds the Fc region of immunoglobulins of many mammalian species with different degrees of binding strenght (see table 1) and with an orientation that allows the $F(ab)_2$ 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.

Example of applications:

- **specific and sterically oriented bond of antibodies**
- **highest specificity and capacity**
- **retains antibody activity and orients antibody for maximum binding**
- **generally not suitable for sandwich ELISA assays**

Product specifications

Available configurations
96-well microplates, solid or with 12 removable 8-well strips.

Coating
Recombinant Protein A/G (M.W. 50.4 kDa) is a fusion protein between Protein A and Protein G. The Protein A portion is from *Staphylococcus aureus* segments E, D, A, B and C and the Protein G portion is from *Streptococcus sp.* segments C1 and C3, expressed in *E. coli*. Protein A/G is coated using 200 µl/well. The strips are post-coated (blocked) for low non specific binding and long-term stability.

Binding capacity
Microplate was saturated with biotinylated human IgG at a concentration of 0.4 – 0.5 µg/ml (400 – 500 ng/well) in an ELISA format using Streptavidin-HRP diluted mixed with Streptavidin as detector and TMB as substrate (see figure 1 for data and experiment details).
The Biomat Protein A/G microplate shows a nominal **binding capacity** falling between **2.66 – 3.33 pmol IgG/well** (100 µl volume)

Uniformity
Microplates show a **CV% less than 10**.

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 the expiration date.

Table 1. Binding affinities of recombinant Protein A, G and A/G for antibodies class.

Species	Antibody Class	Protein A	Protein G	Protein A/G
Human	Total IgG	S	S	S
	IgG_1, IgG_2, IgG_4	S	S	S
	IgG_3	W	S	S
	IgM	W	N	W
	IgD	N	N	N
	IgA	W	N	W
	Fab	W	W	W
	ScFv	W	N	W
Mouse	Total IgG	S	S	S
	IgG_1	W	M	M
	IgG_{2a}, IgG_{2b}, IgG_3	S	S	S
	IgM	N	N	N
Rabbit	Total IgG	S	S	S
Guinea Pig	Total IgG	S	W	S
Rat	Total IgG	W	M	M
	IgG_1	W	M	M
	IgG_{2a}	N	S	S
	IgG_{2b}	N	W	W
	IgG_{2c}	S	S	S
Goat	Total IgG	W	S	S
	IgG_1	W	S	S
	IgG_2	S	S	S
Sheep	IgG	W	S	S
	IgG_1	W	S	S
	IgG_2	S	S	S
Chicken	Total IgY	N	N	N
Hamster	Total IgG	M	M	M
Horse	Total IgG	W	S	S
	IgG(ab)	W	N	W
	IgG(c)	W	N	W
	IgG(T)	N	S	S
Pig	Total IgG	S	W	S
Bovine	Total IgG	W	S	S
	IgG_1	W	S	S
	IgG_2	S	S	S
Dog	Total IgG	S	W	S
Cat	Total IgG	S	W	S
Monkey	Total IgG	S	S	S
Donkey	Total IgG	M	S	S

(The table above gives an overview of binding strengths of protein A, G and A/G to different species and subclasses. S: strong binding; M: medium binding; W: weak binding; N: no binding)

TECHNICAL NOTE

1. Add 100 µl of different concentrations of biotinylated human IgG, (diluted from 0.25 to 8.0 µg/ml) to the wells of Protein A/G coated plate and incubate for 30 minutes at room temperature
2. Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween® 20 (Biomat code 200-3) three times
3. Add 100 µl/well of Streptavidin-HRP diluted 1:30,000 mixed with Streptavidin at 5 µg/ml and incubate for 30 minutes at RT
4. Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween® 20 (Biomat code 200-3) three times
5. Add 100 µl /well of TMB substrate solution (Biomat code 500-1) and incubate 15 minutes at room temperature
6. Stop the substrate reaction by adding 100 µl/well of sulphuric acid 1 N (Biomat code 600-1) and read the optical density values at 450 nm

The data show that a plateau has got starting with a biotinylated human IgG concentration falling between 4.0 and 5.0 µg/ml.
This concentration means the well binding capacity we can express as:

- µg/well = 0.4 – 0.5 (400 – 500 ng/well)
- pmol/well = 2.66 – 3.33 (this result is calculated considering the IgG M.W. = 150 kDa)

Figure 1