PROTEIN G COATED SURFACE

The Biomat product is a 96 well coated microplate with recombinant Protein G and a protein to block non-specific binding sites and to maintain stable activity.
Protein G specifically binds the Fc region of immunoglobulins of many mammalian species with different degrees of binding strenght (see table below) 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 G can securely capture IgG applied directly or as antigen/antibody complexes.

Example of applications:

- **specific and sterically oriented bond of IgG**
- **separation of IgG from other immunoglobulins**
- **separation of antigen-antibodies complexes**
- **isolation and analysis of fusion proteins**

Product specifications

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

Coating
Recombinant Protein G (M.W. 26.1 kDa), from *Streptococcus sp.*, expressed in *E. coli*, 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 G microplate shows a nominal **binding capacity** falling between **2.66 – 3.33 pmol IgG/well** (100 µl volume)

Sensitivity
Biotinylated human IgG was detected at a concentration significantly above background in an ELISA format using streptavidin-HRP as detector and TMB as substrate (see figure 2 for data and experiment details).
The Biomat Protein G microplate shows a **sensitivity of 0.056 ng/well of human IgG**.

Uniformity
Microplates show a **CV% less than 10** when used as a catcher of biotinylated human IgG in an ELISA format using streptavidin-HRP as detector and TMB as substrate.

Table 1. Binding affinities of recombinant Protein A and G for Immunoglobulin binding domains

Species	Ig Subclass	Protein A	Protein G
Human	Total Ig	S	S
	IgG1, IgG2, IgG4	S	S
	IgG3	W	S
	IgD	W	N
	IgA	W	N
	IgE	W	N
	IgM	W	N
Mouse	Total Ig	S	S
	IgG1	W	M
	IgG2a, IgG2b, IgG3	S	S
	IgM	N	N
Rabbit	IgG	S	S
Rat	IgG	N	W-S
Goat	IgG	W-M	M-S
Sheep	IgG	W-M	M-S
Chicken	IgG	N	W
Guinea Pig	IgG	S	W-M
Hamster	IgG	W	M
Horse	IgG	W	S
Pig	IgG	S	W-M
Bovine	IgG	M	S
Dog	IgG	S	W-M
Cat	IgG	S	W

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

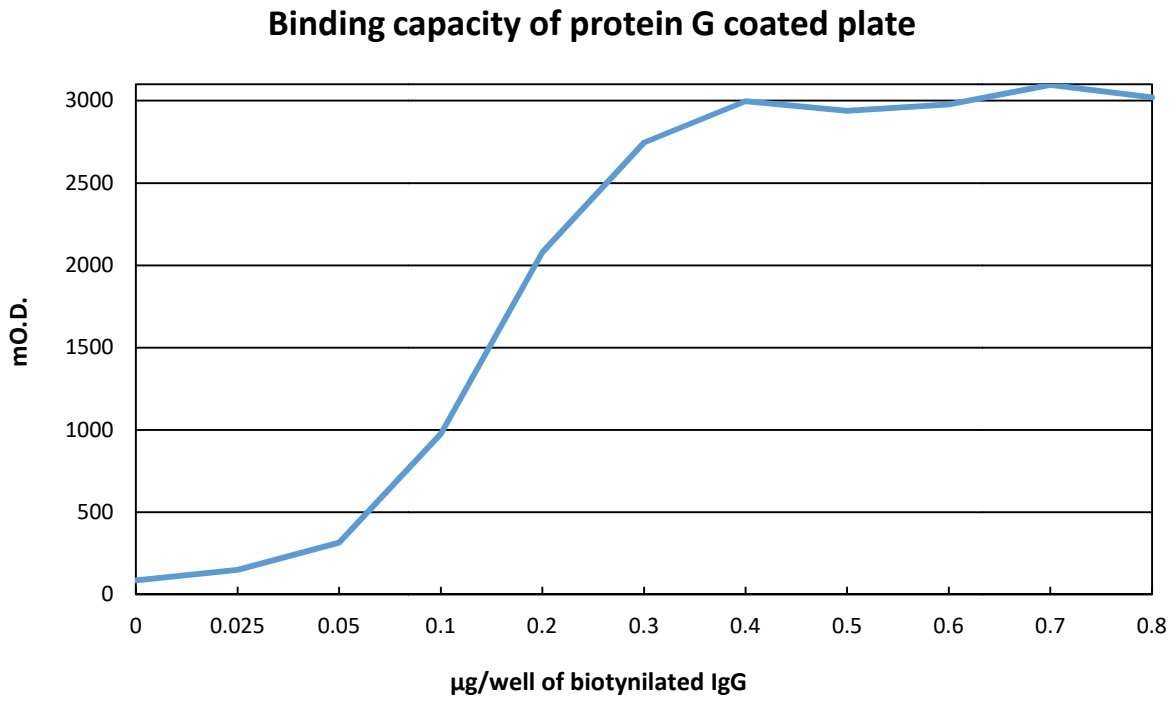
TECHNICAL NOTE N. 26

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 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



TECHNICAL NOTE N. 27

Sensitivity test

1. Add 100 µl of different concentrations of human biotinylated IgG (from 1.56 to 100 ng/ml) to the wells of Protein 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® (Biomat code 200-3) 20 four times
3. Add 100 µl /well of Streptavidin-HRP (BioSpa product code SB01-61, diluted 1:20,000) and incubate for 30 minutes at room temperature
4. Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween® (Biomat code 200-3) 20 four 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 microplate sensitivity was calculated as the lowest biotinylated IgG concentration higher than the mean optical density plus 5 S.D. of 0 ng/ml biotinylated IgG concentration.
Our experiment gave the following results:

- 0 ng/ml biotinylated IgG optical density mean (coming from 8 replicates) = 0.133
- standard deviation = 0.012
- mean + 5 S.D. = 0.193
- sensitivity = 0.056 ng/well of human IgG

Figure 2