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PROTEIN G COATED SURFACES

The Biomat product is a 96 well coated microplate with recombinant Proein 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 (see table 1), 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

Components

Individually pouched 96-well microplates, configured in 12 removable 8-well strips

Coating

Recombinant Protein G (mol. weight 26.1 kDa) ,from Streptococcus sp., expressed in E.coli, is coated using 100 µl/well. The strips are post-coated (blocked) for low non specific binding and long-term stability.

Binding capacity

Microplate was saturated with human IgG at a concentration of 8.0 µg/ml (800 ng/well) in an ELISA format using Streptavidin-HRP as detector and TMB as substrate (see Figure 1 for data and experiment details).

The Biomat Protein G microplate shows a nominal **binding capacity of ~ 5.3 pmol IgG/well**

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 5** when used as a catcher of biotinylated human IgG in an ELISA format using streptavidin-HRP as detector and TMB as substrate.

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 dessicant and use within the expiration date.

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

(The table below 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)

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

TECHNICAL NOTES N. 26 Binding capacity test

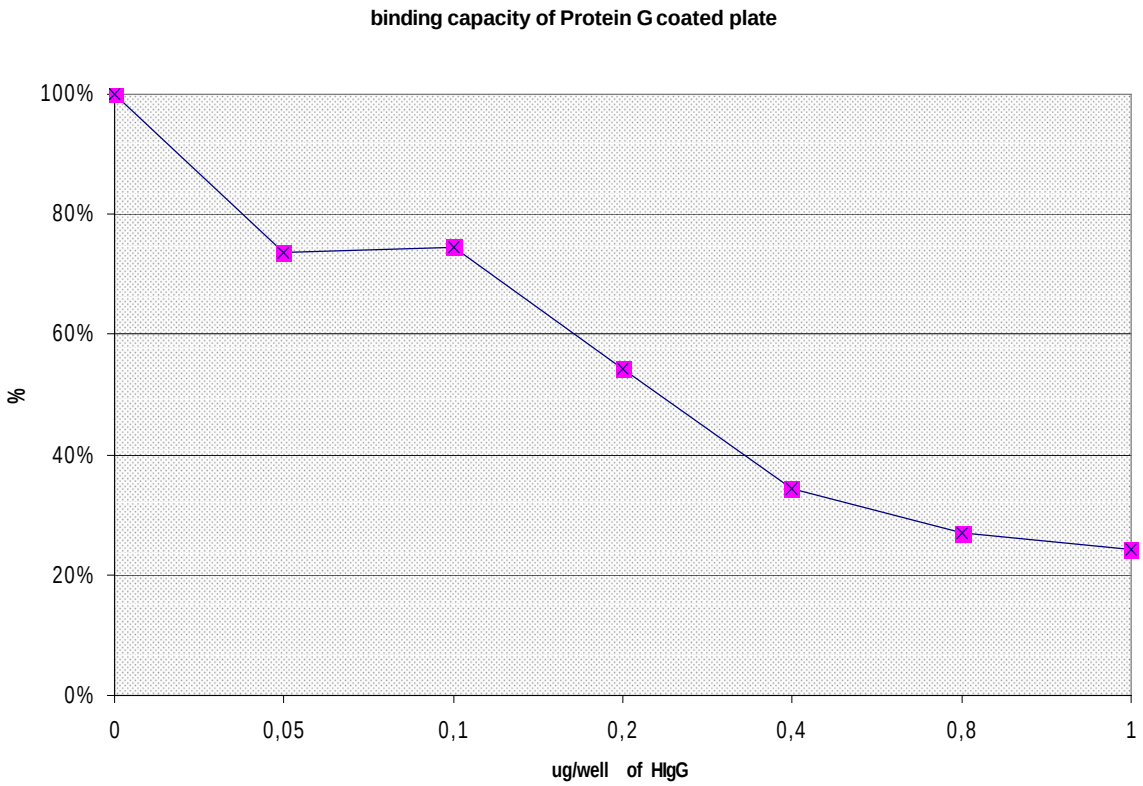
1. Add 100 µl of different concentrations of human IgG (from 0.5 to 10 µg/ml) mixed with a constant amount of human IgG biotinylated (0.01 µg/ml) to the wells of Protein G coated plate and incubate for 30 minutes at room temperature.
2. Empty the wells and wash with 0.1 M PBS pH 7.2,0.05% Tween® 20 four 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® 20 four times
5. Add 100 µl /well of TMB substrate solution and incubate 15 minutes at room temperature.
6. Stop the substrate reaction by adding 100 µl /well of sulphuric acid 1 N and read the optical density values at 450 nm

The data show that a plateau has got starting with an IgG concentration of 8.0µg/ml.

This concentration means the well binding capacity we can express as :

- µg/well = 0.800 (800 ng/well)
- pmol/well= 5.3 (this result is calculated considering the IgG M.W. = 150.000)

Figure 1



TECHNICAL NOTES 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® 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® 20 four times.
5. Add 100 µl /well of TMB substrate solution and incubate 15 minutes at room temperature.
6. Stop the substrate reaction by adding 100 µl /well of sulphuric acid 1 N 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

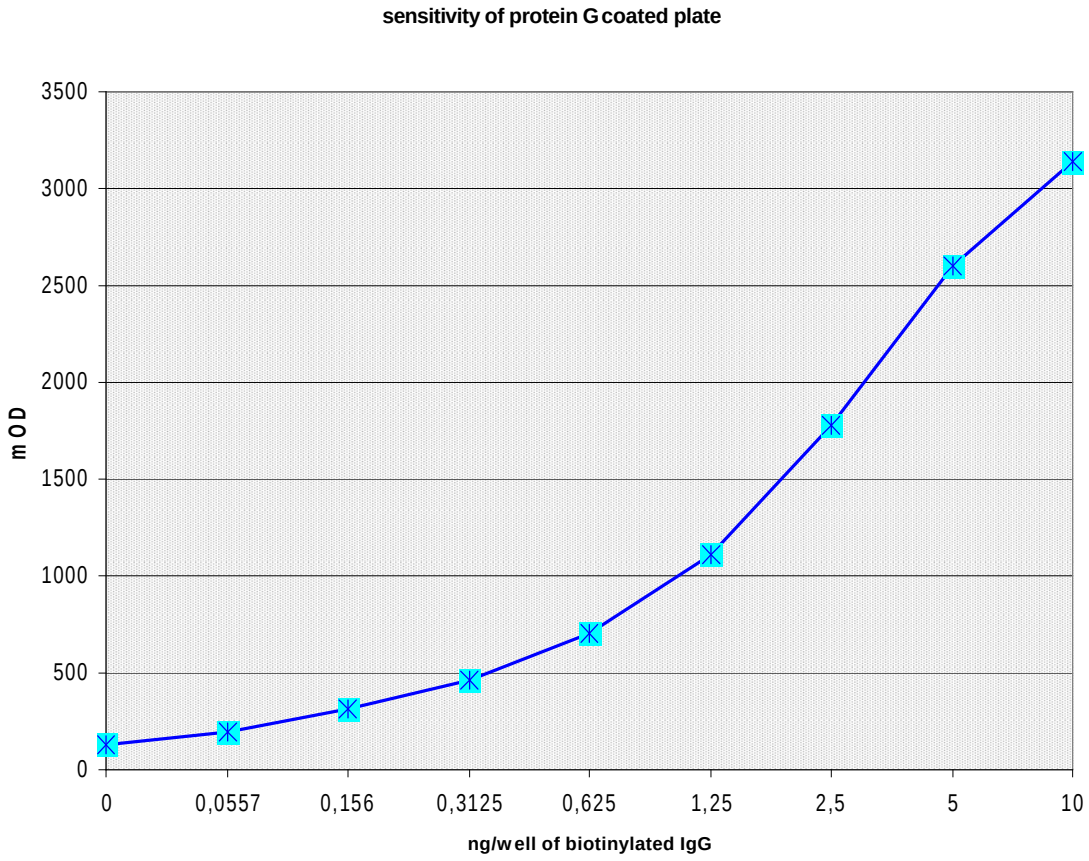


Figure 2