PROTEIN A COATED SURFACE

The Biomat product is a 96 well coated microplate with recombinant Protein A and a protein to block non-specific binding sites and to maintain stable activity.
Protein A 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 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**
- **separation of IgG from contaminants**
- **isolation and analysis of fusion proteins**
- **finding and identifying red cell antibodies (only on U-bottom plates)**

Product specifications

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

Coating
Recombinant Protein A (M.W. 38.9 kDa), from *Staphylococcus aureus subsp. Aureus*, 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 A 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 A microplate shows a **sensitivity of 0.113 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.

Storage and Stability
The microplates, under the indicated storage conditions 2-8 °C, are stable 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 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)

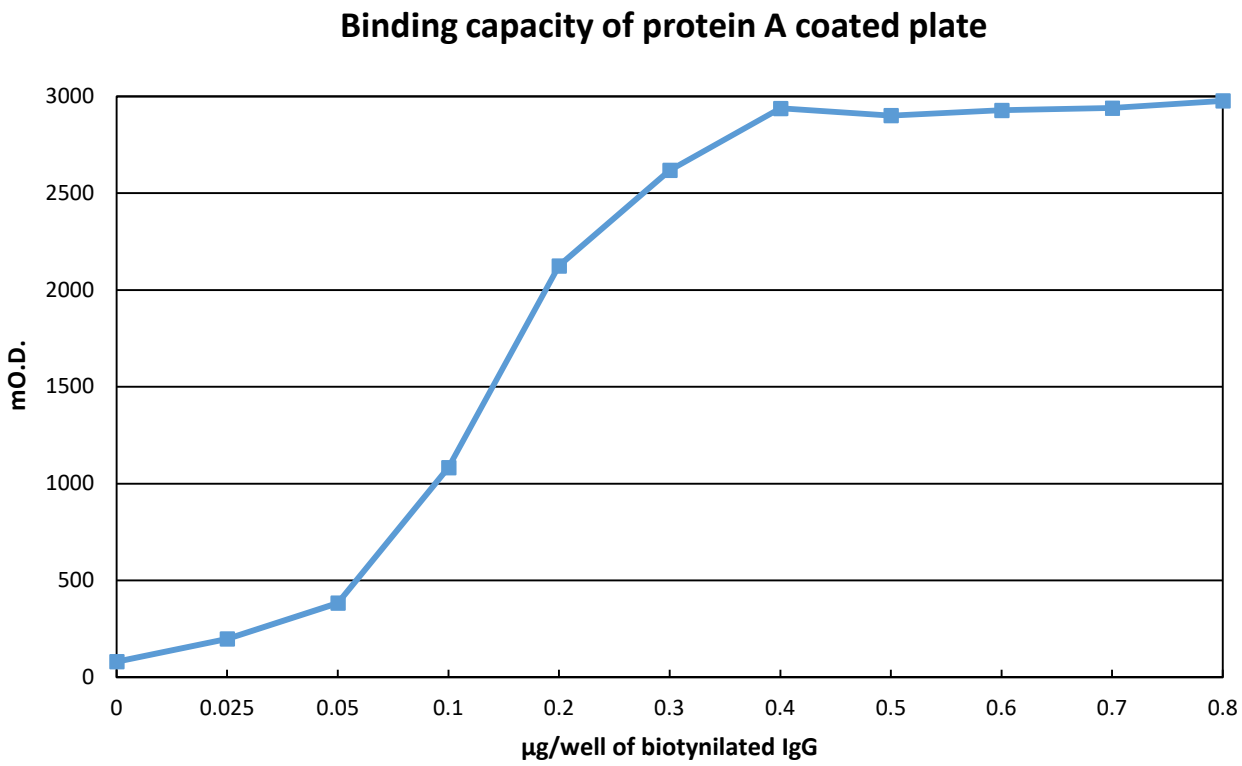
TECHNICAL NOTES N. 23

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 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 NOTES N. 24

Sensitivity test

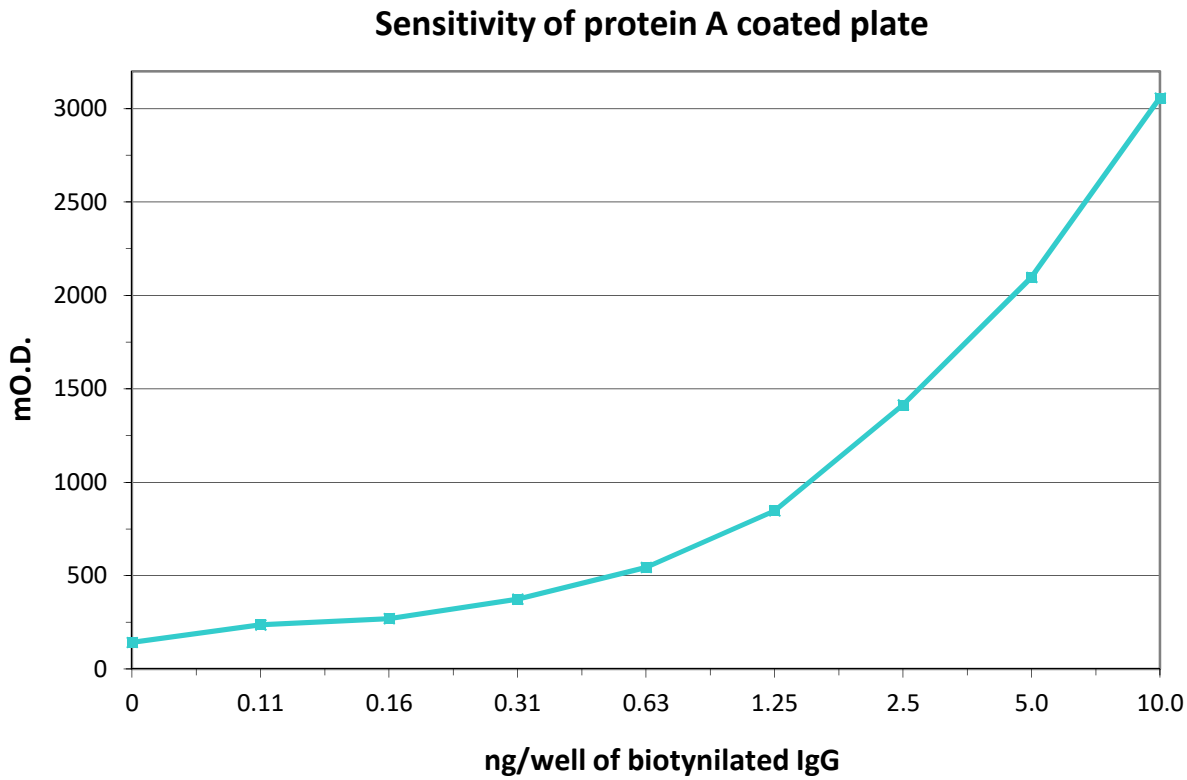
1. Add 100 µl of different concentrations of human biotinylated IgG (from 1.56 to 100 ng/ml) to the wells of Protein A coated plate and incubate for 30 minutes at room temperature
2. Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween® 20 (Biomat code 200-3) four times
3. Add 100 µl /well of Streptavidin-HRP (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 (Biomat code 200-3) 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) = 0.141
- standard deviation = 0.019
- mean + 5 S.D. = 0.236
- sensitivity = 0.113 ng/well of human IgG

Figure 2



TECHNICAL NOTES N. 25

Test for finding and identifying red cell antibodies by means of solid phase method

Protein A microplates U-shape bottom can be adapted to carry out Coombs test.

The two Coombs tests are:

- Direct Coombs test (direct antiglobulin test or DAT)
- Indirect Coombs test (indirect antiglobulin test or IAT)

The direct Coombs test is used in the diagnosis of autoimmune diseases. It detects antibodies bound to the surface of red blood cells. The red blood cells (RBCs) are washed (removing the patient's own serum) and then incubated with antihuman globulin (also known as "Coombs reagent"). If this produces agglutination of RBCs, the direct Coombs test is positive.

The indirect Coombs test is used in prenatal testing of pregnant women, and in testing blood prior to a blood transfusion. It detects antibodies against RBCs that are present unbound in the patient's serum. In this case, serum is extracted from the blood, and then the serum is incubated with RBCs of known antigenicity. If agglutination occurs, the indirect Coombs test is positive.

The two Coombs tests are based on the fact that anti-human antibodies, which are produced by immunizing non-human species with human serum, will bind to human antibodies, commonly IgG or IgM. Animal anti-human antibodies will also bind to human antibodies that may be fixed onto antigens on the surface of RBCs, and in the appropriate test tube conditions this can lead to agglutination of RBCs.

The phenomenon of agglutination of RBCs is important here, because the resulting clumping of RBCs can be visualized; when clumping is seen the test is positive and when clumping is not seen is negative.
The clumping evaluation could be a drawback as it lacks an objective point of termination that can be readily determined automatically or visually in that the reactions that occur within the dilution series vary in intensity to the extent that weak reactions can be incorrectly interpreted as negative for example.

Through the use of a microplate U-shape bottom with Protein A it is easier to carry out a method of cross-sampling, for example.
The erythrocytes are preliminarily incubated with sera or plasma that contain antibodies against erythrocytes, and the coated or uncoated erythrocytes are washed and transferred along with an anti-human reagent to the Protein A microplate.
Erythrocytes coated with antibodies of IgG type are attached by way of anti-human IgG constituent of the reagent to the solid phase in the form a visible film.
Here as well, uncoated erythrocytes form buttons of sedimentary cells.

These two reaction patterns can be read visually or spectrophotometrically to arrive at an objective "yes" or "no"