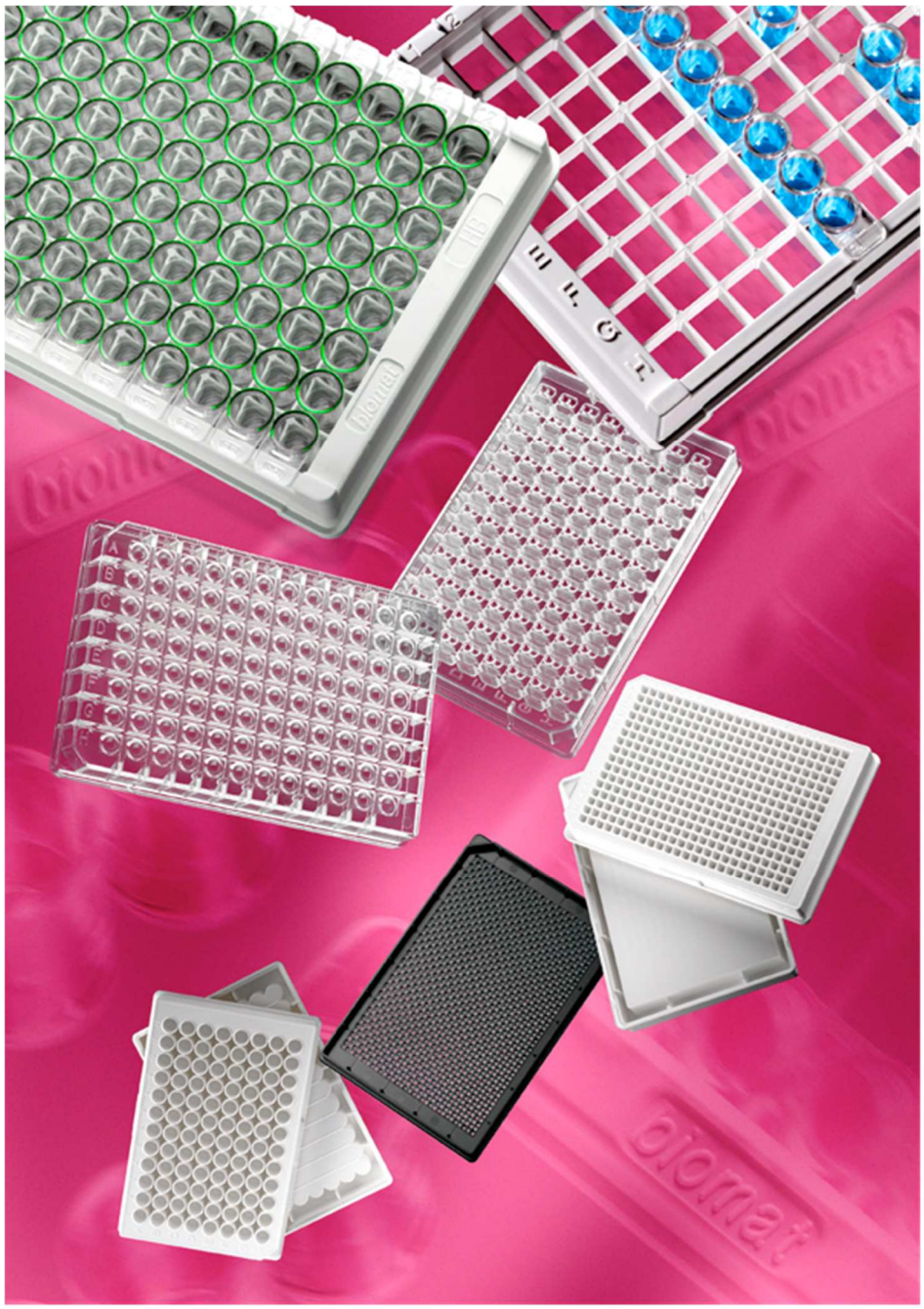
biomat

Your partner for high quality
microplates and special surfaces



Products – Services
Technical Information

THE COMPANY

The company mission is to support life science's manufacturers and researchers producing surface modified plastic articles and developing surface modification services.

Biomat was established in 1992 as an engineering company dedicated to the development of special plastic products (microplates, PCR products, and other products) for diagnostic and medical applications.

The growing need of surfaces capable of binding biological molecules oriented us to focus on the surface modifications employing different technologies: from plasma to bio-coating and our team developed a wide experience in these fields enabling us to offer customized solutions.

In 1995 we started the production of our own microplates for immunoassays with modified surfaces. From the original Medium and High Binding capacity 96 well plates for ELISA, LIA and FIA, we extended our production to different formats and developed several types of surfaces.

Our knowledge of surface modifications, employing different technologies and working on different products, enables us to serve IVD manufacturers and researches operating in the different fields that need surface modified articles: from microplates to Petri dishes, to a wide range of custom-made articles.

IVD manufacturers represent Biomat's main customers, in addition new areas such as molecular diagnostic and genomics are requiring very specific surface properties applied onto different platforms from PS microplates to PP PCR products and are rising new opportunities.

The fields of applications of Biomat's products are:

- ELISA – LIA - FIA
- MOLECULAR BIOLOGY
- HTS
- TISSUE CULTURE

At the same time, the new platforms such as biochips, microarrays or multiple immunoassays, extend the application of the coated/modified surfaces to different applications such as:

- DNA methylation
- Binding of Oligos
- Detection of Exosomes

Throughout the years, Biomat has constantly invested in cutting-edge innovation of its products and services in order to offer the most advanced technologies for new immunoassays and research applications.

For over 20 years Biomat has been trusted worldwide for the quality of its products.

STRATEGY

The biomedical field needs multi-disciplinary expertise, that is the reason why Biomat has built a network of reliable companies capable of supplying top services related to its activity (e.g. surface analysis).

Our experience and skills include many techniques of surface modification: plasma, Gamma irradiation, chemical, biological coating, which are exploited in order to offer the best technology for each application.

Biomat's strategy is based on a flexible structure and to the capacity to adapt it to the technological development and to the expansion of the markets. This is pursued through:

- reliable technical installations with a high degree of automation and easy scalability
- focusing on research in order to keep the pace with the technological development

biomat
Surface modifications - Diagnostic devices - Engineering

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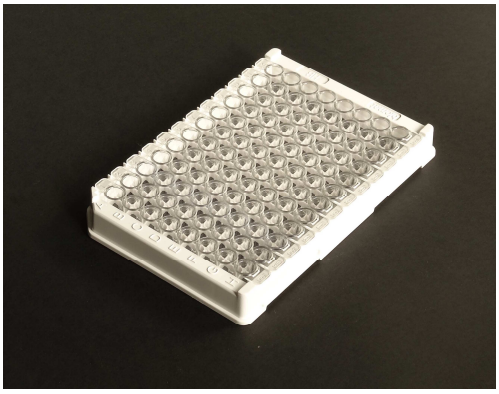
OUR OFFER



PRODUCTS

- IMMUNOASSAY PLATES
- HTS PLATES
- TISSUE CULTURE PLATES
- GLASS BOTTOM PLATES
- PCR PLATES/TUBES

ACCESSORIES

- BARRIER BAGS
- STRIP REMOVER
- SILICA GEL

SERVICES

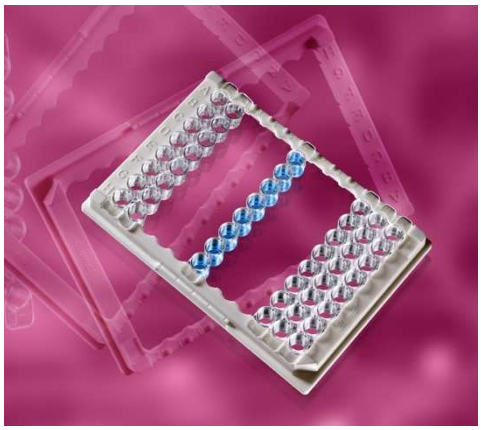
- COATING AND SURFACE MODIFICATION
- PRODUCT DEVELOPMENT
- SURFACE ANALYSIS

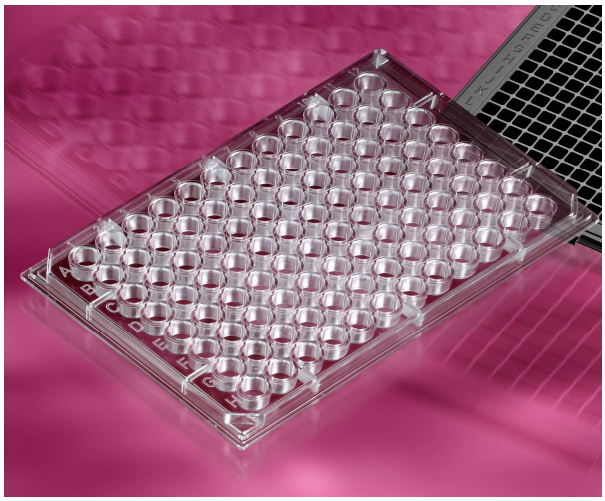
IMMUNOASSAY PLATES

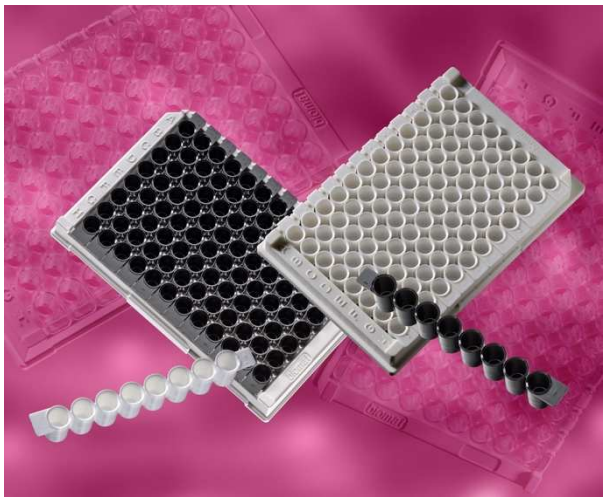
Biomat 's full range of 96 well immunoassay plates includes both solid and 8 well strip format assembled on 12 x 8 and single well holding frame (breakable strips), allowing the maximum flexibility for the user.
Made in **Transparent, White** and **Black** Polystyrene the plates can be used for ELISA, Luminescence and Fluorescence assays.

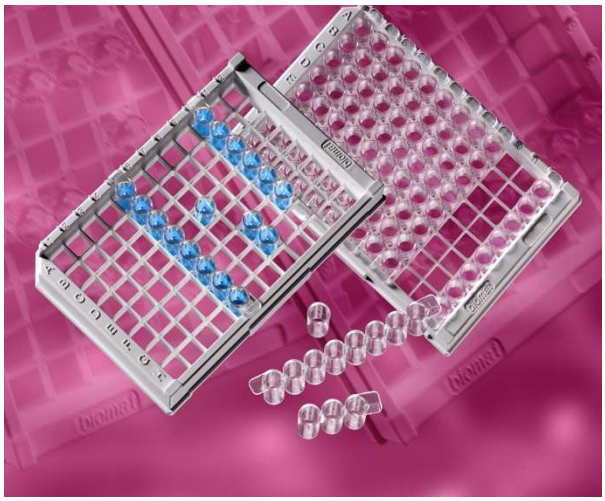
Their design offers the best performances:

- manufactured in pure polystyrene with low fluorescence
- the optical quality, important to reduce the background signal, is pursued through the mould design
- the radius edged inner bottom of the wells improves the efficiency of washings
- the external lid warrants vertical alignment when using single wells
- a rim protects the external face of the bottom from scratches
- the plates comply with SBS standards and the design assures a good performance in automatic processing plant







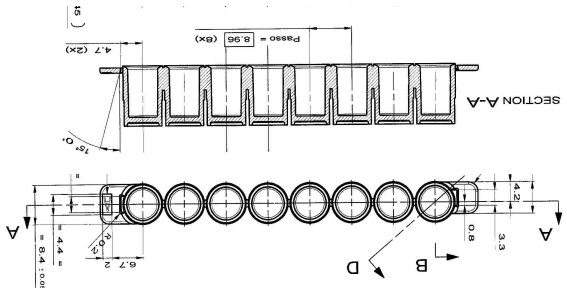


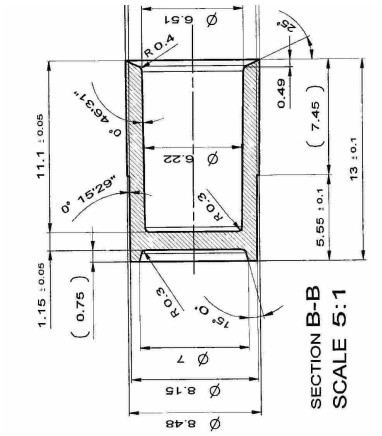
TECHNICAL FEATURES

96 wells strip plates are offered with different well capacities:

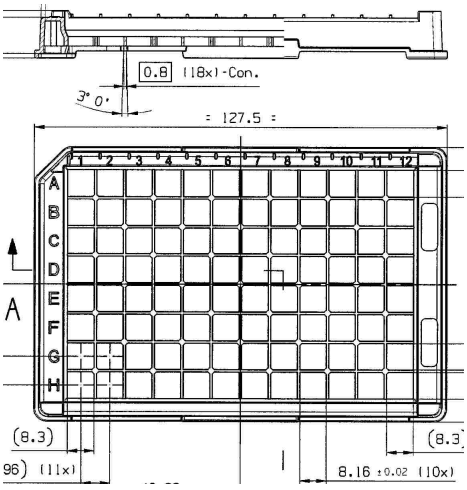
- **350µl**
- **360µl**
- **400µl**

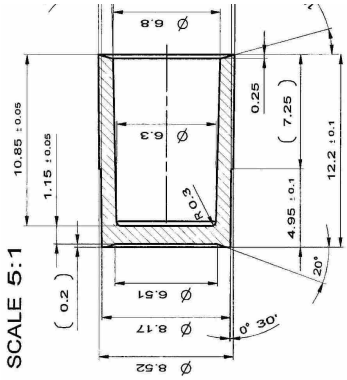
The general features of the wells are the same and all fit on the same frames.



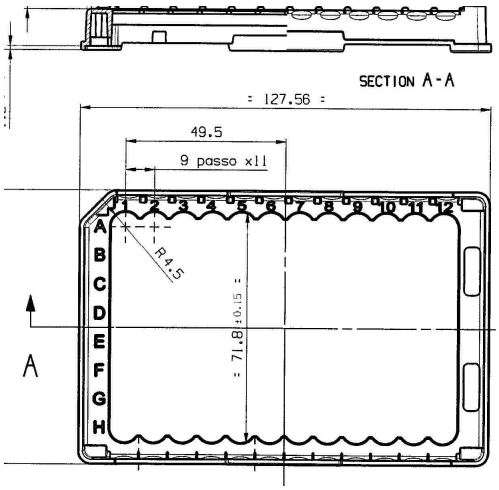


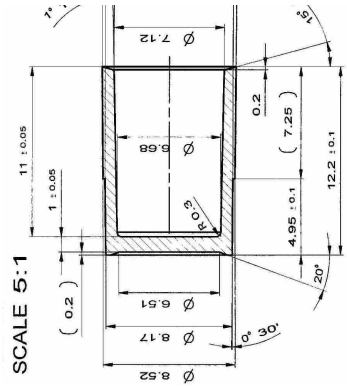
350µl





360µl





400µl

TECHNICAL TIPS

Since the behaviour and performance of the microplates are directly related to some physical dimensions it can be useful to have some data immediately available.

WELL SURFACE

The area which is covered by the liquid is that which is involved in the reactions.
At each level the total area is made of the bottom surface, which is the same for every level, and the wall surface, which is variable.

OPTICAL PATH

The optical path is another important feature, since it can affect the reading, sometimes giving a false impression of higher or lower sensitivity.
A smaller diameter of the well means that the level of the same quantity of liquid will be higher so that the light beam will pass through a wider extension of coloured liquid. Obviously some other factors as:

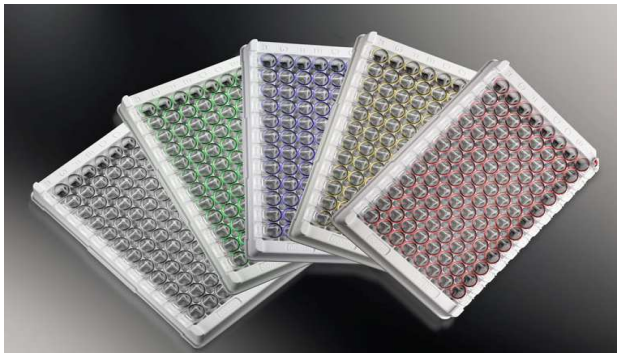
- the shape of the meniscus of the liquid
- the exact alignment of the light beam so that it passed at the very center of the well which is the low or higher, depending upon the wettability of the well
- the wettability itself affects this value, so these figures are intended only to be indicative.

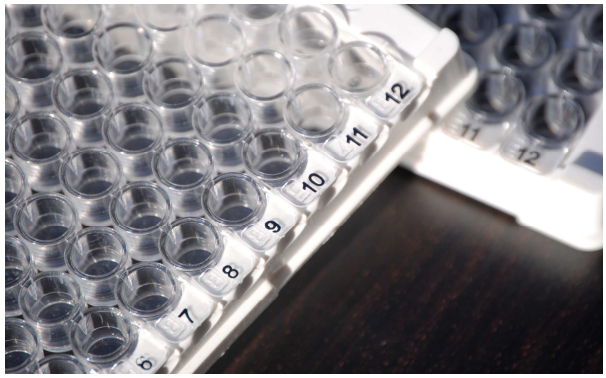
Level µl	Optical path mm	Bottom surface mm²	Wall surface mm²	Total surface mm²
100	2,98	30,68	61,27	91,95
200	5,98	30,68	117,37	148,05
300	8,97	30,68	176,05	206,73

CUSTOMISED PLATES

CODED PLATES

Color and number coded strips are available on every type of surface and are customizable to customer's needs.
Color is marked on the upper rims enabling fast and reliable identification of the strip or of the single well.
Each microplate is marked with one color only.
Number codes are marked on the edge of the strip.
The codes are applied by heat transfer.





The plates are tested before and after the marking in order to warrant that no modification of their properties occurred during the treatment.

BARCODED PLATES

Plan and barcode labels are applied on one or more sides of the plates, according with customer's need.

CUSTOMISED PLATES (OEM)

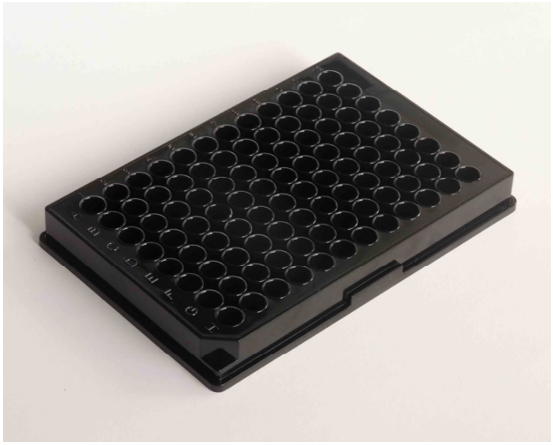
Customers' own brand name can be inserted on the frames. Minimum quantity order is required.
Lot reservation is possible.

HTS PLATES

Pharmaceutical industry relies extensively on High Throughput Screening (HTS) for drug-discovery.
Automation is one keyword in this field: HTS assays are mostly performed on 96, 384 and 1536 well plates.
HTS too evolved its interest towards coated surfaces that proved to be useful for the most advanced drug-like compounds, ligands for receptors, enzymes, ion-channels or other pharmaceuticals.

Biomat offers both 96 and 384 well plates for High Throughput Screening (HTS).
The plates are manufactured in pure polystyrene with low fluorescence.

- The dimensions conform to SBS standards and the design assure precise and fast handling with most of the automatic systems
- The pigment widely incorporated within the polymer warrants low crosstalk
- White, black and clear plates are available for the different techniques



STANDARD SURFACES

A wide range of surfaces offers the proper instrument for the needs of every user. These surfaces are available on Immunoassay Plates and HTS Plates.

Available standard surfaces are:

Medium binding capacity	A hydrophobic surface suitable for passive adsorption of proteins with different grades of hydrophobicity.
High binding capacity	A hydrophilic surface suitable for passive adsorption of proteins with different grades of hydrophilicity.
No binding capacity	A surface that prevents the binding of proteins to the wells. Addressed to those procedures that need to avoid any modification of the activity of the molecules (e.g. enzymes) induced by the reactions that could occur with the well surface.

FEATURED COATED SURFACES

Biotin	Biotin Coated Plates can be used in immunoassays with avidin, streptavidin, neutravidin or other biotin-binding proteins
Streptavidin **Streptavidin HB** **Neutravidin**	Streptavidin, Streptavidin HB and Neutravidin Coated Plates can be used in immunoassays for binding antibodies or probes for ELISA and other target-specific assays
Protein A **Protein G** **Protein A/G**	Protein A, G and A/G Coated Plates provide alternative to direct, passive adsorption methods for immobilizing antibodies for ELISA and other plates-based assay techniques
Concanavalin A **Jacalin** **Wheat Germ**	Lectin Coated Plates can be used in assays for binding carbohydrate-binding proteins and glycoproteins
Poly-D-Lysine **Poly-L-lysine** **Poly-L-Arginine**	Polyamino acids Coated Plates can be used to exploit their high density of α amino, α carboxyl, ε amino, guanidine groups to react through electrostatic and stereospecific bonds
Calmodulin	Calmodulin Coated Plates can be used in assays for binding specific proteins mainly with hydrophobic sites in their surface
Heparin catcher	Heparin Binding Coated Plates can be used to immobilize heparin without having to modify the molecule in some way
Amine (primary amine) **Carboxyl** **Maleimide**	The plates modified with Amine or Carboxylic groups and Maleimide Coated can be used for binding peptides or other molecules through covalent specific bonds
Secondary Antibodies	• Goat anti-mouse IgG or • Goat anti-rabbit IgG can be used for binding antibodies when they are available in low quantities, denaturated and even when they are present in an impure protein samples.

Customised surfaces are developed jointly with the customer in order to optimize the kit's performance.

Each type of surface is tested in order to guarantee the binding capacity:

- stability
- uniformity
- reproducibility

MEDIUM BINDING CAPACITY

A hydrophobic surface suitable for passive adsorption of proteins with different grades of hydrophobicity.

FEATURES

Assays in which the adsorbed molecule present large hydrophobic regions, such as antibodies.

Furthermore this surface is highly selective and shows high affinity towards hydrophobic polypeptides that present a molecular weight > 10kDa.

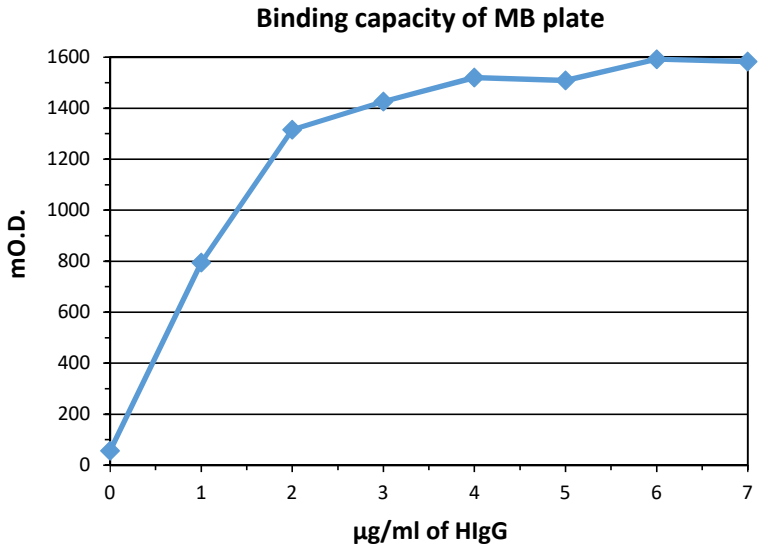
PERFORMANCES

A coating of antibodies of IgG class was carried out to evaluate its usefulness along with to evaluate the binding capacity of this surface.

Method 15

Method 15 is an indirect method with human IgG coated on medium binding plates and then revealed through an Anti-HIgG-HRP conjugate.

1. dispense 100 µl/well of different concentrations of human IgG diluted in 0.1M PBS pH 7.2: 1-2-3-4-5 µg/ml and incubate overnight at 4°C
2. wash 3 times with 0.1M PBS pH 7.2 + 0.05%Tween® 20
3. dispense 150 µl/well of BSA 1% in 0.1M PBS pH 7.2 and incubate 2 hours at R.T. for blocking the remaining active sites
4. wash 3 times with 0.1M PBS pH 7.2+ 0.05% Tween® 20
5. dispense 100 µl/well of Goat Anti-HIgG-HRP conjugate and incubate 30' at R.T.
6. wash 3 times with 0.1M PBS pH 7.2 + 0.05% Tween® 20
7. dispense 100 µl/well of TMB
8. after 30' stop the reaction with H_2SO_4 1 N
9. reading is made at 450 nm



The data show that a plateau has got starting with an IgG concentration of 2.0 µg/ml.

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

- µg/well = 0.200 (200 ng/well/100µl)

As 100 µl of liquid, in term of area, represent 1 cm^2 it is possible to state that the binding capacity is close to 200 ng/ cm^2. These data are well correlated with other experiments carried out with an unmodified polystyrene surface.

HIGH BINDING CAPACITY

TECHNICAL NOTE N. 10

HB 8

A hydrophilic surface suitable for passive adsorption of proteins with different grades of hydrophilicity.

FEATURES

Assays in which the adsorbed molecule exceeds (up to 400 ng/cm^2) the complementary molecules that have to be detected.
Furthermore this surface is highly selective and shows high affinity towards the adsorption of molecules also when those are present in very small amounts (<50 ng/cm^2) allowing to obtain the maximum sensitivity of the test.

APPLICATION

Protein - Antibody Anti-IgM adsorbed onto solid phase used in IgM capture assays
Lipo-protein - Rubella antigen adsorbed onto solid phase used in IgG assays
ELISA (competitive) tests for Steroid Hormones and TSH

PERFORMANCES

A test simulating a competitive method shows the performance of this surface.

Principle

A limited amount of biotinylated albumin, coated on the well surface, was allowed to react with a constant amount of streptavidin peroxidase along with various amounts of unlabelled streptavidin used as standard solutions. Unbound reagents were rinsed away
After incubation with TMB and stopping by adding sulphuric acid, the colour intensity was read at 450 nm.

Calculation of results

The enzymatic activity, present in the well, is inversely proportional to the concentration of unlabelled streptavidin present in the standard solution.
Table 1 shows the records of the absorbance (mO.D.) at 450 nm for each point of standard solution.

Table 1

	HB 8	B standard/B Max x 100	Competitor	B standard/B Max x 100
B Max	1327	100	1287	100
B 5 ng/ml	1093	82.4	1129	87.7
B 10 ng/ml	921	69.4	898	69.8
B 25 ng/ml	644	48.5	627	48.7
B 50 ng/ml	424	31.9	421	32.7
B 100 ng/ml	267	20.1	264	20.5
B 200 ng/ml	131	9.9	170	13.2

The maximum binding reactivity (B Max) is represented by the absorbance derived from streptavidin-peroxidase in the presence of 0 ng of unlabelled streptavidin.
The presence of unlabelled streptavidin in the standard solutions is expressed using a percentage ratio between the relative absorbance of that standard solution (B standard concentration) and the absorbance derived from streptavidin-peroxidase in the presence of 0 ng of unlabelled streptavidin.

TECHNICAL NOTE N. 4

Comparison of different types of High Binding capacity polystyrene strips

In order to check the performances of different polystyrene strips' surfaces we performed an extensive study comparing Biomat High Binding Capacity (HB8) strips with one of the most used High Binding Capacity type of strips currently available on the market.
To ensure the validity of results the test was performed with methods as near as possible to the standard methods which, in our knowledge, are used by manufacturers when preparing polystyrene strips as solid phase to set up diagnostic kits (e.g. ToRCH ELISA kits).

The kind of molecules used for testing were both IgM and IgG for determination of Rubella, Cytomegalovirus and Toxoplasma.

Materials and methods

I Preparation of plates

Antigens for Rubella, Cytomegalovirus and a polyclonal antibody Rabbit IgG to Human IgM (DAKO A426) were diluted in carbonate-bicarbonate buffer 0.1 M pH 9.6 and both samples of strips were coated at the same time. The coating was performed at 4° C.
After a washing step the plates were saturated with PBS 0.1 M pH 7.2 containing 1% Bovine Serum Albumin and incubated overnight at 4°C.
After a further washing step the plates were dried at 37°C for two hours, then sealed under vacuum and stored at 4°C until use.
All the sera used in this test came from hospital laboratories and were certified to be positive or negative using the commercial kits manufactured by: Behring; Biomerieux-Vidas; Sorin Biomedica.

II IgG assay

The scheme for performing the IgG assays was the following:

1. 100µl diluted samples and calibrators were incubated for 30 min at room temperature in each type of antigen- coated wells
2. a washing step with 0.1M PBS pH 7.2 + 0.05% Tween® 20 was performed
3. 100µl/well of purified goat-anti Human Fc IgG Peroxidase were added and incubated for 30 min at room temperature
4. a further washing step as that at point 2. was performed
5. 100µl/well of substrate (TMB) were added and incubated for 15 min at room temperature
6. the reaction was stopped by adding 100 µl of sulphuric acid
7. reading at 450 nm was then performed

III IgM capture assay

The scheme for performing the IgM assays was the following:

1. 100µl diluted samples and calibrators were incubated for 1 hour at room temperature in the common anti-IgM coated wells
2. a washing step with 0.1M PBS pH 7.2 + 0.05% Tween® 20 was performed
3. 100µl/well of a complex of the appropriate biotinylated purified antigen and streptavidin-peroxidase was added and incubated for 1 hour at room temperature
4. a further washing step as that at point 2. was performed
5. 100µl/well of substrate (TMB) were added and incubated for 30 min at room temperature
6. the reaction was stopped by adding 100 µl of sulphuric acid
7. reading at 450 nm was then performed

ANALYSIS OF DATA

The data obtained from the two types of samples of microplates were processed in the following way:

NEGATIVE SAMPLES	**POSITIVE SAMPLES**
a. min. O.D. observed for each type of strip	a. coefficient of correlation
b. Max. O.D. observed for each type of strip	b. linear regression calculated as $y = a + bx$ (1)
c. mean of Standard Deviations	with a confidence level of 95% x values were those obtained with competitor's samples y values were those obtained with Biomat HB8 samples

The results are exposed in the following tables

TABLE A IgG assays

IgG assay to Cytomegalovirus		
TOTAL SERA TESTED	51	
NEGATIVE SERA	32	
POSITIVE SERA	19	
calibrators A.U. /ml	O.D. comp.	O.D. biomat
120	2,393	2,272
50	1,375	1,463
20	0,748	0,891
10	0,322	0,38
0	0,015	0,012
A.U.=Arbitrary Unit		
O.D. of negative sera	comp.	biomat
minumum	0,031	0,043
Maximum	0,183	0,173
mean of Standard Deviation	0,111+-0,035	0,109+-0,030
results of positive sera		
R=	0.994	
y=	2,644+0,996x	

IgG assay to Rubella		
TOTAL SERA TESTED	81	
NEGATIVE SERA	46	
POSITIVE SERA	35	
calibrators I.U. /ml	O.D. comp.	O.D. biomat
250	3,367	2,971
75	1,833	1,624
25	0,718	0,609
8	0,251	0,246
0	0,015	0,016
I.U.=International Unit		
O.D. of negative sera	comp.	biomat
minumum	0,035	0,027
Maximum	0,192	0,119
mean of Standard Deviation	0,076 +- 0,032	0,061 +- 0,023
results of positive sera		
R=	0,961	
y=	4,567 + 0,948 x	

TABLE B IgM assays

IgM assay to Cytomegalovirus		
TOTAL SERA TESTED	32	
NEGATIVE SERA	23	
POSITIVE SERA	9	
calibrators	O.D. comp.	O.D. biomat
HIGH POSITIVE	2,092	1,732
LOW POSITIVE	0,65	0,558
NEGATIVE	0,146	0,14
O.D. of negative sera	comp.	biomat
minumum	0,153	0,142
Maximum	0,431	0,36
mean of Standard Deviation	0,189+-0,06	0,181+-0,04
results of positive sera		
R=	0,975	
y=	0,24+0,84 x	

IgM assay to Toxoplasma Gondii		
TOTAL SERA TESTED	58	
NEGATIVE SERA	27	
POSITIVE SERA	31	
calibrators	O.D. comp.	O.D. biomat
HIGH POSITIVE	2,673	2,353
LOW POSITIVE	0,931	0,665
NEGATIVE	0,225	0,17
O.D. of negative sera	comp.	biomat
minumum	0,228	0,174
Maximum	0,847	0,594
mean of Standard Deviation	0,484+-0,186	0,38+-0,131
results of positive sera		
R=	0,978	
y=	0,047+0,88 x	

The above results are confirmed by the correspondence with the clinical data obtained by the hospital laboratory

DISCUSSION OF RESULTS

The analysis of the data exposed in tables A and B shows:

- a comparable binding capacity of proteins of both Biomat HB8 and competitor's strips
- the capacity of both types of samples to assure the specific binding between the coated protein and the protein to be revealed: 100% of results of our tests (on 232 sera, 94 positive and 128 negative) were confirmed stating the sensitivity and specificity of both types of samples with all the tested sera
- the result of regression analysis, whose acceptable value had been fixed at $R \geq 0.95$ has been fully respected
- the ranges of coefficients and values obtained in equation (1):

a which must not significantly differ from 0

And

b whose values ranged from $0.8 \leq b \leq 1.2$

proved the strict correspondence of results.

NO BINDING CAPACITY

TECHNICAL NOTE N. 11

A surface that prevents the binding of proteins to the wells. Addressed to those procedures that need to avoid any modification of the activity of the molecules (e.g. enzymes) induced by the reactions that could occur with the well surface.

Here the test carried out for checking the properties of our **No Binding** capacity when IgG were coated on its surface. In parallel was carried out a test using, as comparison, the HB binding surface to demonstrate the natural protein adsorption.

Method 15

1. dispense 100 µl/well of 5 µg/ml of human IgG diluted in 0.1M PBS pH 7.2 and incubate overnight at 4°C
2. wash 3 times with 0.1M PBS pH 7.2 + 0.05%Tween® 20
3. dispense 150 µl/well of BSA 1% in 0.1M PBS pH 7.2 and incubate 2 hours at R.T. for blocking the remaining active sites
4. wash 3 times with 0.1M PBS pH 7.2+ 0.05% Tween® 20
5. dispense 100 µl/well of Goat Anti-HIgG-HRP conjugate and incubate 30' at R.T.
6. wash 3 times with 0.1M PBS pH 7.2 + 0.05% Tween® 20
7. dispense 100 µl/well of TMB
8. after 15' stop the reaction with H_2SO_4 1 N
9. reading is made at 450 nm
10.

	NO binding	**High binding HB8**
m O.D. values	62	1563

The results clearly demonstrate that no IgG adsorption happens in NO binding wells.

COATED SURFACES

BIOTIN

Biotin, or vitamin H (M.W. 244 Da), is a small naturally occurring cofactor that is present in every living cell in very minute amounts (usually less than 0.0001 %). The biotin molecule normally exists bound to proteins (such as pyruvate carboxylase) through its valeric acid carboxylic group by an amide bond to lysine side-chain amines.
Biotin coated surfaces offer a powerful instrument to carry out one of the most useful interactions in immunochemistry that involves the specificity binding of biotin to avidin, streptavidin or neutravidin.
This binding shows a great constant affinity (10^{-15} M).

The polystyrene optical features don't change, allowing the modified surface to be used as a valid tool to carry out biological tests.

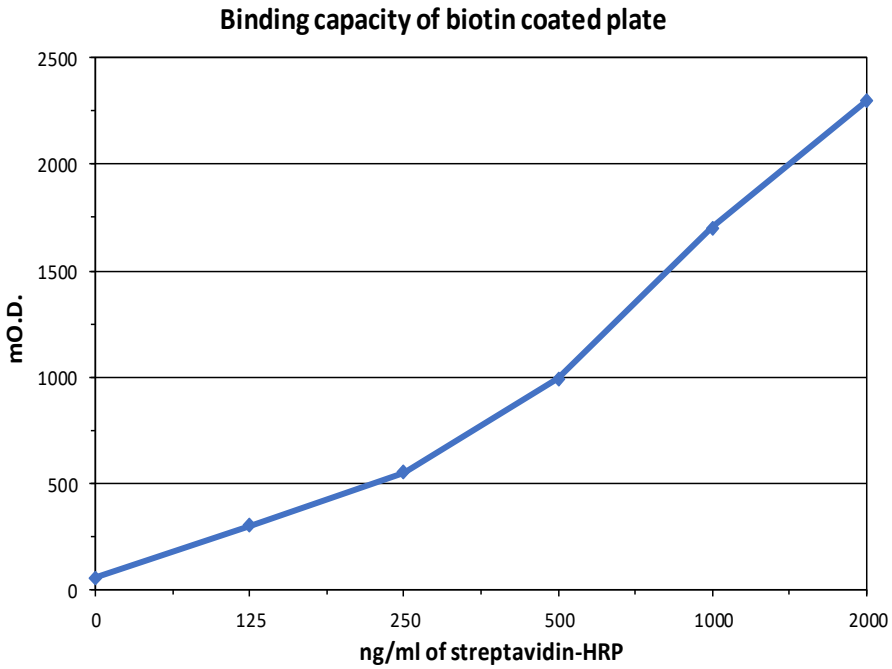
This surface shows its usefulness for these applications:

- interactions with avidin
- interactions with streptavidin
- interaction with neutravidin

TECHNICAL NOTE N. 20

Evaluation of binding specificity towards Streptavidin-peroxidase conjugate

1. Dilute streptavidin-peroxidase conjugate from 2000 to 125 ng/ml with 0.1 M PBS pH 7.2 containing 0.2 % BSA
2. Add 100 µl of each dilution to the wells of Biotin coated plate and incubate 60 minutes at room temperature
3. Leave blank wells as control
4. Empty the wells and wash with 0.1M PBS pH 7.2 + 0.05% Tween® 20 four times
5. Add 100 µl/well of TMB substrate solution and incubate 10 minutes at room temperature
6. Stop the substrate reaction by adding 100 µl of sulphuric acid 1 N and read the optical density values at 450 nm



CALMODULIN

Biomat has developed a polystyrene surface with physically adsorbed Calmodulin protein. The Calmodulin Ca^{++} binding protein is able to bind proteins mainly with hydrophobic sites in its surface.
The polystyrene optical features don't change, allowing the modified surface to be used as a valid tool to carry out biological tests.

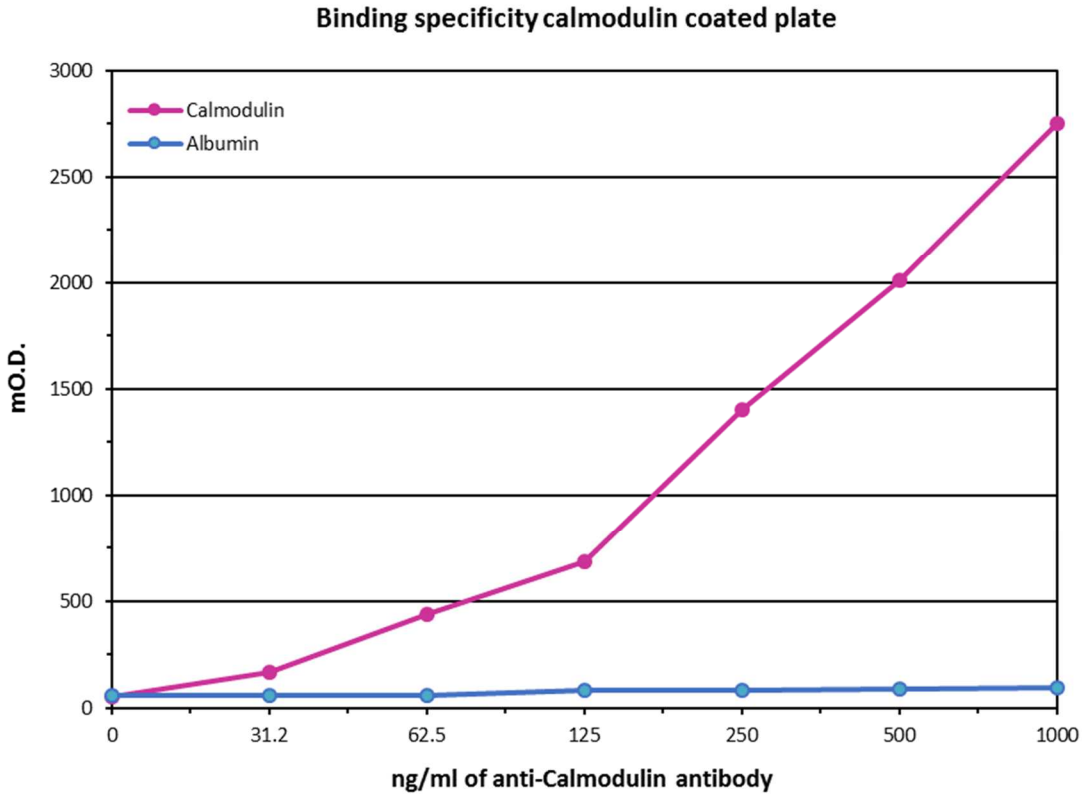
This surface shows its usefulness for these applications:

- interactions with proteins involved in glycogen metabolism
- interactions with factors involved in neurotransmission mechanism
- interactions with enzymes involved in the NAD*/NADP* phosphorylation system

TECHNICAL NOTE N. 19

Evaluation of binding specificity towards monoclonal anti-calmodulin

1. Dilute anti-calmodulin monoclonal antibody from 1000 to 0 ng/ml with 0.1 M PBS pH 7.2 containing 0.2 % BSA
2. Add 100µl of each dilution to the wells of Calmodulin coated plate and incubate 60 minutes at room temperature. Add the same solutions to Albumin coated plate as comparison for evaluate the specificity of binding
3. Leave blank wells as control
4. Empty the wells and wash with 0.1M PBS pH 7.2 + 0.05% Tween® 20 four times
5. Add 100 µl/well of conjugate goat anti-mouse-HRP and incubate 30 minutes at room temperature
6. Add 100 µl/well of TMB substrate solution and incubate 10 minutes at room temperature
7. Stop the substrate reaction by adding 100 µl of sulphuric acid 1 N and read the optical density values at 450 nm



CONCANAVALIN A

Concanavalin A, belonging to the lectins family, is a Hemagglutinin from the common jack bean *Canavalia ensiformis*. It is well known that lectins have been used extensively for the isolation of glyco-conjugates and glycoproteins with specific carbohydrate structures.
Concanavalin A shows specific affinity for molecules containing α-D-mannopyranosyl, α-D-glucopyrannosyl and sterically related.
Concanavalin A coated surfaces offer a powerful and sensitive instrument for binding in specific way the carbohydrate fraction of glycoproteins, enzymes and cell membranes.
The optical properties of polystyrene remain unchanged, allowing to use the modified surface as powerful tool for diagnostic assays.

Example of applications:

- interaction with glycoproteins, glycopeptides and enzyme-antibody conjugates
- polysaccharides and glycolipids
- interaction with cellular membranes, hormones and hormone receptors

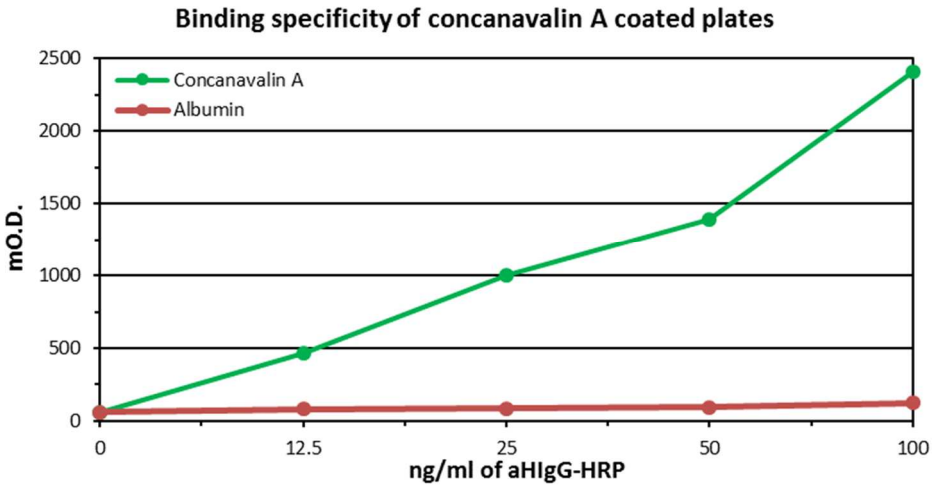
TECHNICAL NOTE N. 15

General procedure for binding a biomolecule to Concanavalin A coated surface

1. dilute biomolecule (sample) to 0.5-5 μg/ml in an appropriate neutral pH buffer (Buffer should contain 1mM Ca^{++} and 1mM Mn^{++}; in fact these ions promote the interaction between saccharide groups and Concanavalin A coated surface)
2. proceed with incubation: conditions depend on biomolecule structure
3. wash four times to remove the unbound material
4. proceed with your specific test:
   - to point out the bound biomolecule
   - to use the bound biomolecule to point out a specific counter molecule

Example of test: binding specificity of Concanavalin A coated plates

1. Dilute AHIgG-HRP from 100 ng/ml to 12.5 ng/ml in pure distilled water containing 1 mM $CaCl_2 \cdot 2\ H_2O$ + 1 mM $MnCl_2 \cdot 4\ H_2O$
2. Add 100μl of each solution to the wells of Concanavalin A coated plate and incubate 30 minutes at room temperature. Add the same solutions to albumin coated plate as comparison for evaluate the specificity of binding
3. Leave blank wells as control
4. Empty the wells and wash with 0.1M PBS pH 7.2 + 0.05% Tween® 20 four times
5. Add 100 μl /well of TMB substrate solution and incubate 10 minutes at room temperature
6. Stop the substrate reaction by adding 100 μl of sulphuric acid 1 N and read the optical density values at 450 nm



HEPARIN CATCHER

Background

Heparin is one of the most intensively studied glycosaminoglycans as a result of its anticoagulant properties.
Heparin is used as an anticoagulant either in its native unfractionated form (UFH) MW ~ 16 kDa or as partially depolymerized form called low molecular weight (LMW) heparin MW ~ 4-8 kDa.

Heparin assay

Biomat developed three Heparin Catcher plates, as special surfaces onto which heparin at different ranges of U/ml can be immobilized.
The proposed assays exploit the different Biomat heparin catcher plates, by quantitative enzyme-linked assays for the *in vitro* measurement of unfractionated heparin in low protein content fluid such as a buffer.
These heparin ELISA tests are competitive assays in which the colorimetric signal is inversely proportional to the amount of heparin present in the sample.

Principle of the assay

Samples to be assayed are at first mixed with a known amount of biotinylated heparin within the wells of Biomat heparin catcher plate.
The Heparin in the sample competes with biotinylated heparin to bind to the binding sites of heparin catcher plate.
After the removal of the unbound reagent and sample, a streptavidin-peroxidase conjugate is added to reveal the reaction.
The concentration in the sample is determined using a standard curve of known amounts of heparin.

Typical assay suitable for measuring Heparin (UHF) in saline buffer, in the range 0.01 to 2.0 U/ml

Reagents

1. Biomat heparin catcher plate code **HC1**
2. UFH (Sigma code H 4784, lot 019K1487 140 USP units/mg)
3. Heparin biotin (Sigma code B 9806, lot 128K1599)
4. Streptavidin peroxidase (BioSpa code SB01-61 at 1 mg/ml, lot 00255/1-2)
5. Streptavidin peroxidase diluent
6. TMB Substrate
7. Stop Solution, 0.3 N H_2SO_4
8. Wash Buffer (0.1 M PBS pH 7.2+0.05% Tween® 20)
9. Standard Diluent (0.1 M PBS pH 7.2)

Reagent preparation

1. Heparin Standards: Make dilutions of UFH standards using the Standard Diluent to obtain standards of 0.01, 0.05, 0.1, 0.5,1.0, 2.0 U/ml, starting from UFH H 4784 **(Standardization should be performed using heparin that is the same heparin type contained in your unknowns)**
2. Heparin biotin: Make a 1.25 μg/ml using the Standard Diluent
3. Streptavidin-peroxidase conjugate: Make a 1:25.000 dilution in Streptavidin peroxidase diluent just before the use

Assay Procedure

We suggest to run in duplicate both the heparin standards and samples in order to get the best results.

1. Place the desired number of Biomat heparin catcher coated strips into the holder
2. Dispense 50 μl of Standard Diluent as 0 U/ml standard, heparin standards from 0.01 to 2.0 U/ml and samples into the appropriate wells; immediately after dispense 50 μl of diluted heparin biotin 1.25 μg/ml into each well.
   Moreover add two wells where to dispense 100 μl of Standard Diluent to be used as NSB (non specific binding)
3. Mix well and incubate for two hours at room temperature
4. Remove liquid from the wells and wash three times with 300 μl of 0.1 M PBS pH 7.2
   Blot on absorbance paper or paper towel.
5. Dispense 100 μl of streptavidin-peroxidase conjugate to each well and incubate for 1 hour at room temperature.
6. Remove streptavidin-peroxidase conjugate from all wells. Wash wells three times with 300 μl of Wash Buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 μl of TMB substrate and incubate 15 minutes at room temperature
8. Add 100 μl of Stop solution

9. Read O.D. at 450 nm using an ELISA reader. A dual wavelength is recommended with reference filter of 600-650 nm

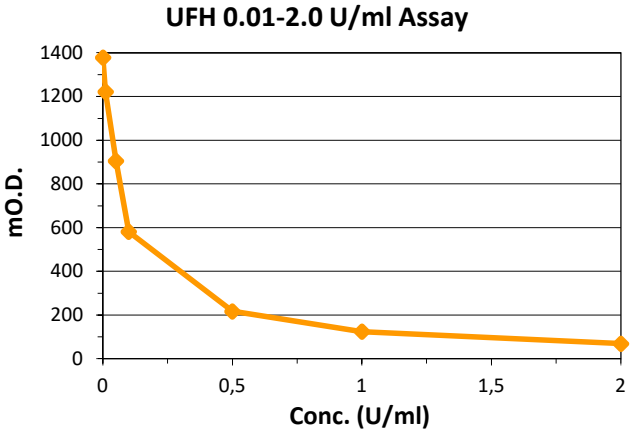
Calculation of results

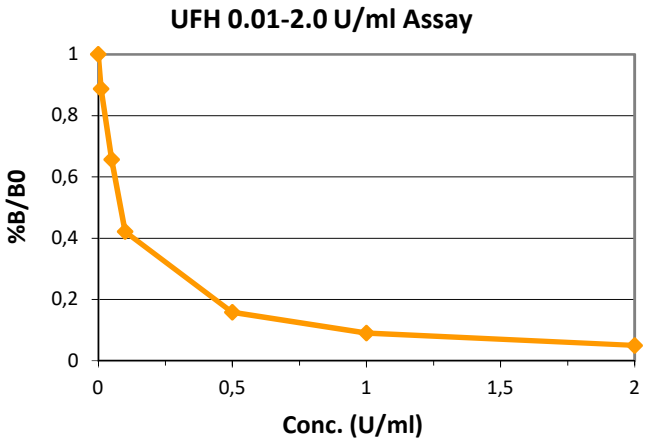
Calculate the mean of the duplicate readings for each standard and sample and subtract the mean NSB optical density.
Construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a linear x-axis and draw the best fit curve through the points on the graph.
% B/B_0 can be calculated by dividing the corrected O.D. for each standard and sample by the corrected 0 U/ml O.D. standard (B_0) and multiplying by 100.
Calculate the concentration of heparin corresponding to the mean absorbance or the % B/B_0 from the standard curve.

Typical Data

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

U/ml	Average mO.D.	Corrected	% B/B_0
NSB	72	-	-
0 (B_0)	1450	1378	100
0.01	1294	1222	88.7
0.05	978	906	65.7
0.1	653	581	42.2
0.5	289	217	15.8
1.0	196	124	9.0
2.0	141	69	5.0





<u>Typical assay suitable for measuring Heparin (UHF) in saline buffer, in the range 0.5 to 40.0 U/ml</u>

Reagents

1. Biomat heparin catcher plate code **HC2**
2. UFH (Sigma code H 4784, lot 019K1487 140 USP units/mg)
3. Heparin biotin (Sigma code B 9806, lot 069K1378)
4. Streptavidin peroxidase (BioSpa code SB01-61 at 1mg/ml, lot 00256/1-1)
5. Streptavidin peroxidase diluent
6. TMB Substrate
7. Stop Solution, 0.3 N H_2SO_4
8. Wash Buffer (0.1 M PBS pH 7.2+0.05% Tween® 20)
9. Standard Diluent (0.1 M PBS pH 7.2)

Reagent preparation

1. Heparin Standards: Make dilutions of UFH standards using the Standard Diluent to obtain standards of 0.5, 2.5, 5.0, 10.0, 20.0, 40.0 U/ml, starting from UFH H 4784. **(Standardization should be performed using heparin that is the same heparin type contained in your unknowns)**
2. Heparin biotin: Make a 2.77 μg/ml using the Standard Diluent
3. Streptavidin-peroxidase conjugate: Make a 1:25.000 dilution in Streptavidin peroxidase diluent just before the use

Assay Procedure

We suggest to run in duplicate both the heparin standards and samples in order to get the best results.

1. Place the desired number of Biomat heparin catcher coated strips into the holder
2. Dispense 10 μl of Standard Diluent as 0 U/ml standard, heparin standards from 0.5 to 40.0 U/ml and samples into the appropriate wells; immediately after dispense 90 μl of diluted heparin biotin 2.77 μg/ml into each well
   Moreover add two wells where to dispense 100 μl of Standard Diluent to be used as NSB (non specific binding)
3. Mix well and incubate for two hours at room temperature
4. Remove liquid from the wells and wash three times with 300 μl of 0.1 M PBS pH 7.2
   Blot on absorbance paper or paper towel
5. Dispense 100 μl of streptavidin-peroxidase conjugate to each well and incubate for 1 hour at room temperature.
6. Remove streptavidin-peroxidase conjugate from all wells. Wash wells three times with 300 μl of Wash Buffer. Blot on absorbance paper or paper towel
7. Dispense 100 μl of TMB substrate and incubate 15 minutes at room temperature
8. Add 100 μl of Stop solution
9. Read O.D. at 450 nm using an ELISA reader. A dual wavelength is recommended with reference filter of 600-650 nm

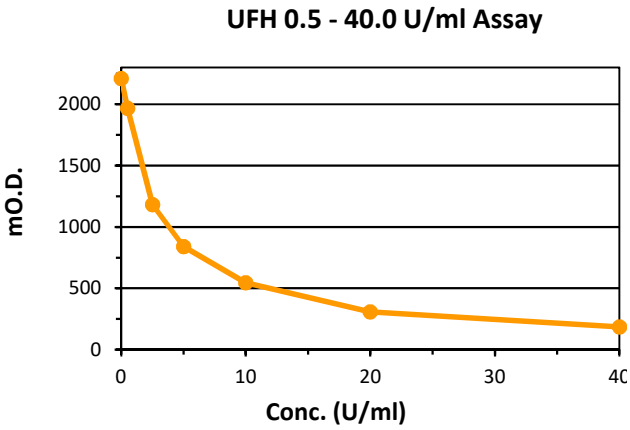
Calculation of results

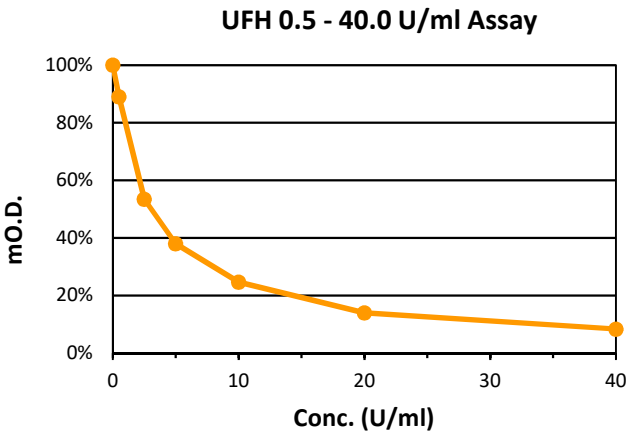
Calculate the mean of the duplicate readings for each standard and sample and subtract the mean NSB optical density.
Construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a linear x-axis and draw the best fit curve through the points on the graph.
% B/B_0 can be calculated by dividing the corrected O.D. for each standard and sample by the corrected 0 U/ml O.D. standard (B_0) and multiplying by 100.
Calculate the concentration of heparin corresponding to the mean absorbance or the % B/B_0 from the standard curve.

Typical Data

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

U/ml	Average mO.D.	Corrected	% B/B_0
NSB	116	-	-
0 (B_0)	2326	2210	100%
0.5	2082	1966	89.0%
2.5	1298	1182	53.5%
5.0	956	840	38.0%
10.0	661	545	24.7%
20.0	425	309	14.0%
40.0	301	185	8.4%





Typical assay suitable for measuring Heparin (UHF) in saline buffer, in the range 2.0 to 160.0 U/ml

Reagents

1. Biomat heparin catcher plate code **HC3**
2. UFH (Sigma code H 4784, lot 019K1487 140 USP units/mg)
3. Heparin biotin (Sigma code B 9806, lot 069K1378)
4. Streptavidin peroxidase (BioSpa code SB01-61 at 1mg/ml, lot 00256/1-1)
5. Streptavidin peroxidase diluent
6. TMB Substrate
7. Stop Solution, 0.3 N H_2SO_4
8. Wash Buffer (0.1 M PBS pH 7.2+0.05% Tween® 20)
9. Standard Diluent (0.1 M PBS pH 7.2)

Reagent preparation

1. Heparin Standards: Make dilutions of UFH standards using the Standard Diluent to obtain standards of 2.0, 10.0, 20.0, 40.0, 80.0, 160.0 U/ml, starting from UFH H 4784. **(Standardization should be performed using heparin that is the same heparin type contained in your unknowns)**
2. Heparin biotin: Make a 5.0 µg/ml using the Standard Diluent
3. Streptavidin-peroxidase conjugate: Make a 1:25.000 dilution in Streptavidin peroxidase diluent just before the use

Assay Procedure

We suggest to run in duplicate both the heparin standards and samples in order to get the best results.

1. Place the desired number of Biomat heparin catcher coated strips into the holder
2. Dispense 10 µl of Standard Diluent as 0 U/ml standard, heparin standards from 2.0 to 160.0 U/ml and samples into the appropriate wells; immediately after dispense 200 µl of diluted heparin biotin 5.0 µg/ml into each well

Moreover, add two wells where to dispense 200 µl of Standard Diluent to be used as NSB (non specific binding)

3. Mix well and incubate for two hours at room temperature
4. Remove liquid from the wells and wash three times with 300 µl of 0.1 M PBS pH 7.2

Blot on absorbance paper or paper towel

5. Dispense 200 µl of streptavidin-peroxidase conjugate to each well and incubate for 1 hour at room temperature
6. Remove streptavidin-peroxidase conjugate from all wells. Wash wells three times with 300 µl of Wash Buffer Blot on absorbance paper or paper towel.
7. Dispense 200 µl of TMB substrate and incubate 15 minutes at room temperature
8. Add 100 µl of Stop solution
9. Read O.D. at 450 nm using an ELISA reader. A dual wavelength is recommended with reference filter of 600-650 nm

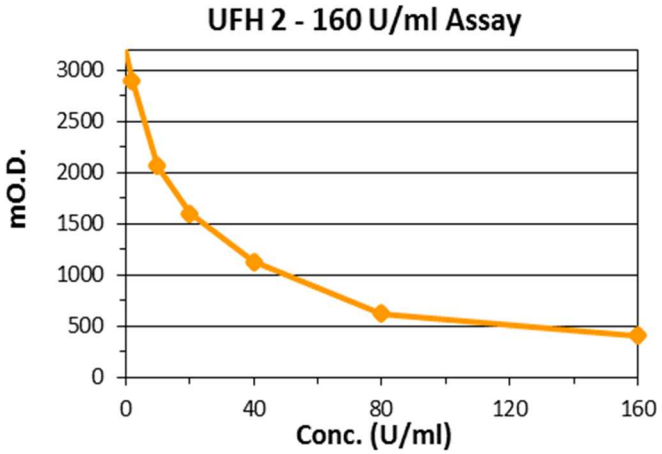
Calculation of results

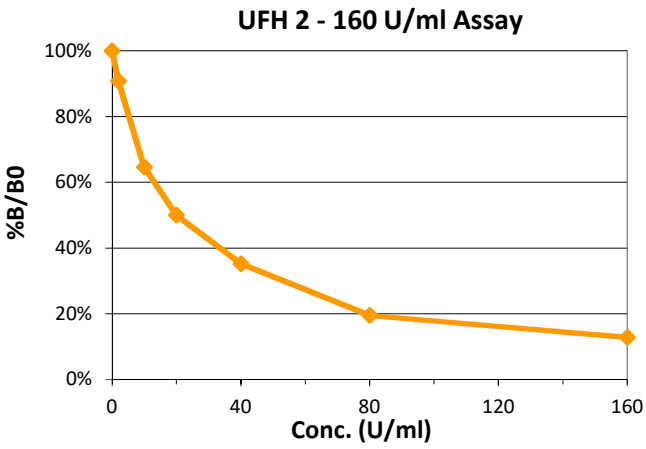
Calculate the mean of the duplicate readings for each standard and sample and subtract the mean NSB optical density.
Construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a linear x-axis and draw the best fit curve through the points on the graph.
% B/B_0 can be calculated by dividing the corrected O.D. for each standard and sample by the corrected 0 U/ml O.D. standard (B_0) and multiplying by 100.
Calculate the concentration of heparin corresponding to the mean absorbance or the % B/B_0 from the standard curve.

Typical Data

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

U/ml	Average mO.D.	Corrected	% B/B_0
NSB	167	--	--
0 (B_0)	3368	3201	100%
2	3072	2905	90.8%
10	2236	2069	64.6%
20.0	1766	1599	50.0%
40.0	1294	1127	35.2%
80.0	791	624	19.5%
160.0	577	410	12.8%





JACALIN

Biomat Jacalin 96 Well Microplates are coated in combination with a surfactant to block non-specific binding sites and to maintain stable activity.
Jacalin, belonging to the lectins family, is a tetrameric (M.W. 40 kDa) carbohydrate-binding protein isolated from the seeds of jack fruit *Artrocarpus integrifolia* agglutinin. It is well known that lectins have been used extensively for the isolation of glycol-conjugates and glycoproteins with specific carbohydrate structures.
Jacalin shows specific affinity for molecules containing non reducing α-D-galactosyl groups, usually present in the biochemical structure of IgA1 and cell membranes.

Example of applications:

- Human IgA1 specific binding, sterically oriented
- Purification of human immunoglobulins (especially IgA1)
- Separation of immunocomplexes antigen-antibody
- Separation of IgA1 from contaminants
- Stimulation of T-cells

Product specifications

Coating

Jacalin (M.W. 40 kDa), from the seeds of jack fruit *Artrocarpus integrifolia*, 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 biotinylated human IgA at a concentration of 6 µg/ml (600 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 Jacalin microplate shows a nominal **binding capacity of ~ 4 pmol IgA/well**.

Sensitivity

Biotinylated human IgA 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 Jacalin microplate shows a **sensitivity of 0.0161 µg/ml (1.61 ng/well) of IgA**.

Uniformity

Microplates show a **CV% less than 5** when used as a catcher of biotinylated human IgA 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 desiccant and use within the expiration date.

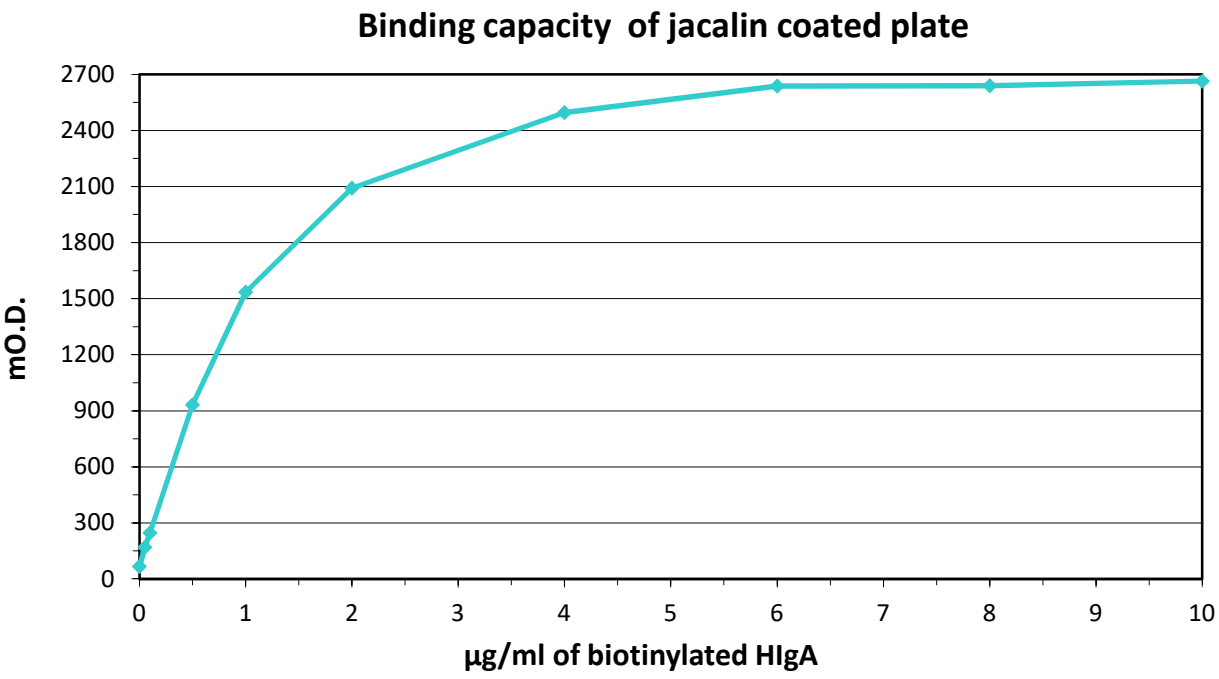
TECHNICAL NOTE N. 28

Binding capacity test

1. Add 100 µl of different concentrations of biotinylated human IgA (from 0.05 to 10 µg/ml) to the wells of Jacalin coated plate diluted in pure distilled water containing 1 mM $CaCl_2 \bullet 2\ H_2O$ + 1 mM $MnCl_2 \bullet 4\ H_2O$. Incubate for 60 minutes at room temperature
2. Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween 20® four times Add 100 µl /well of Streptavidin-HRP (BioSpa product code SB01-61 at 1 mg/ml), diluted 1:20.000 in pure distilled water containing 1 mM $CaCl_2 \bullet 2\ H_2O$ + 1 mM $MnCl_2 \bullet 4\ H_2O$) and incubate for 30 minutes at room temperature
3. Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween 20® four times
4. Add 100 µl /well of TMB substrate solution and incubate 5 minutes at room temperature
5. Stop the substrate reaction by adding 100 µl/well of sulphuric acid 0.3 N and read the optical density values at 450 nm.

The data show that a plateau has got starting with a biotinylated human IgA concentration of 6 µg/ml.
This concentration means the well binding capacity we can express as:
µg/well = 0.6 (600 ng/well)
pmol/well= 4.0 (this result is calculated considering the IgA1 M.W. = 150 kDa)

Figure 1



TECHNICAL NOTE N. 29

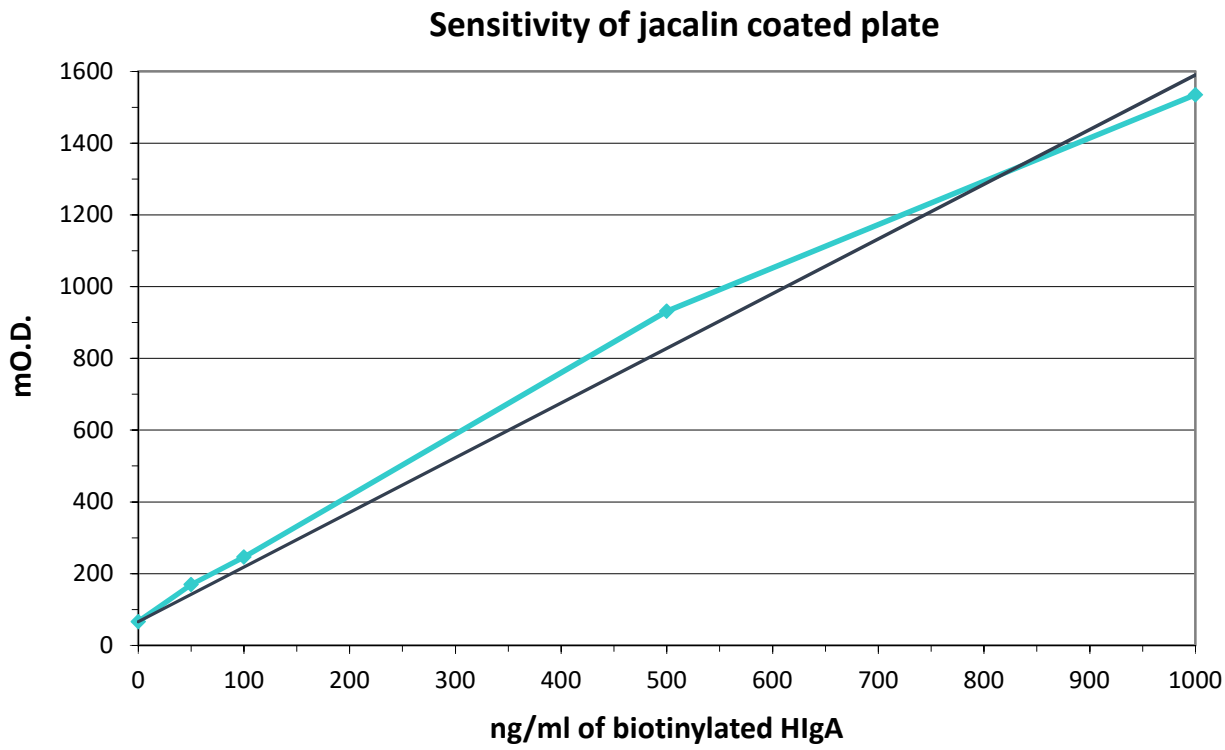
Sensitivity test

1. Add 100 µl of different concentrations of biotinylated human IgA (from 0.05 to 10 µg/ml) to the wells of Jacalin coated plate diluted in pure distilled water containing 1 mM $CaCl_2$•2 H_2O + 1 mM $MnCl_2$•4 H_2O and incubate for 60 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 at 1 mg/ml), diluted 1:20.000 in pure distilled water containing 1 mM $CaCl_2$•2 H_2O + 1 mM $MnCl_2$•4 H_2O) 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 5 minutes at room temperature
6. Stop the substrate reaction by adding 100 µl /well of sulphuric acid 0.3 N and read the optical density values at 450 nm

The microplate sensitivity was calculated as the lowest biotinylated IgA concentration higher than the mean optical density plus 5 S.D. of 0 µg/ml biotinylated IgA concentration.
Our experiment gave the following results:

- 0 µg/ml biotinylated IgA optical density mean (coming from 4 replicates) = 0.0655
- standard deviation = 0.0050
- mean + 5 S.D. = 0.0905
- sensitivity = 0.0161 µg/ml (1.61 ng/well) of biotinylated human IgA

Figure 2



MALEIMIDE

The Biomat product is a 96 well coated microplate with maleimide and treated to block non-specific binding sites and to maintain stable activity.
Maleimide coated surfaces offer a powerful instrument for binding biomolecules containing free sulfhydryl groups (e.g. peptides that contain a terminal cysteine or thiol containg haptens), or reducibile disulfide bonds that are difficult to coat onto polystyrene plates. These coated plates are a very useful tool for assays requiring site-directed orientation of particular biomolecules especially during antibody production.
At pH 6.5-7.5 maleimide reacts with free sulfhydryl groups to yeld stable bonds, while the reaction with amine becomes significant at pH > 7.5.
If sulfhydryl-containing peptides and proteins oxidize in solution and form disulfide bonds, they must be preventively reduced to free sulfhydryls for allowing interaction with maleimide.

Product specifications

Coating
A derived maleimide 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 Glutathione Ethyl Ester Biotin Amide (BioGEE) at a concentration of 2.5 µg/ml (250 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 Maleimide microplate shows a nominal binding capacity of **~ 440 pMol BioGEE/well**.

Uniformity
Microplates show a **CV% less than 5** when used as a catcher of Glutathione Ethyl Ester Biotin Amide (BioGEE) 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 desiccant and use within the expiration date.

TECHNICAL NOTE N. 35

Binding capacity test

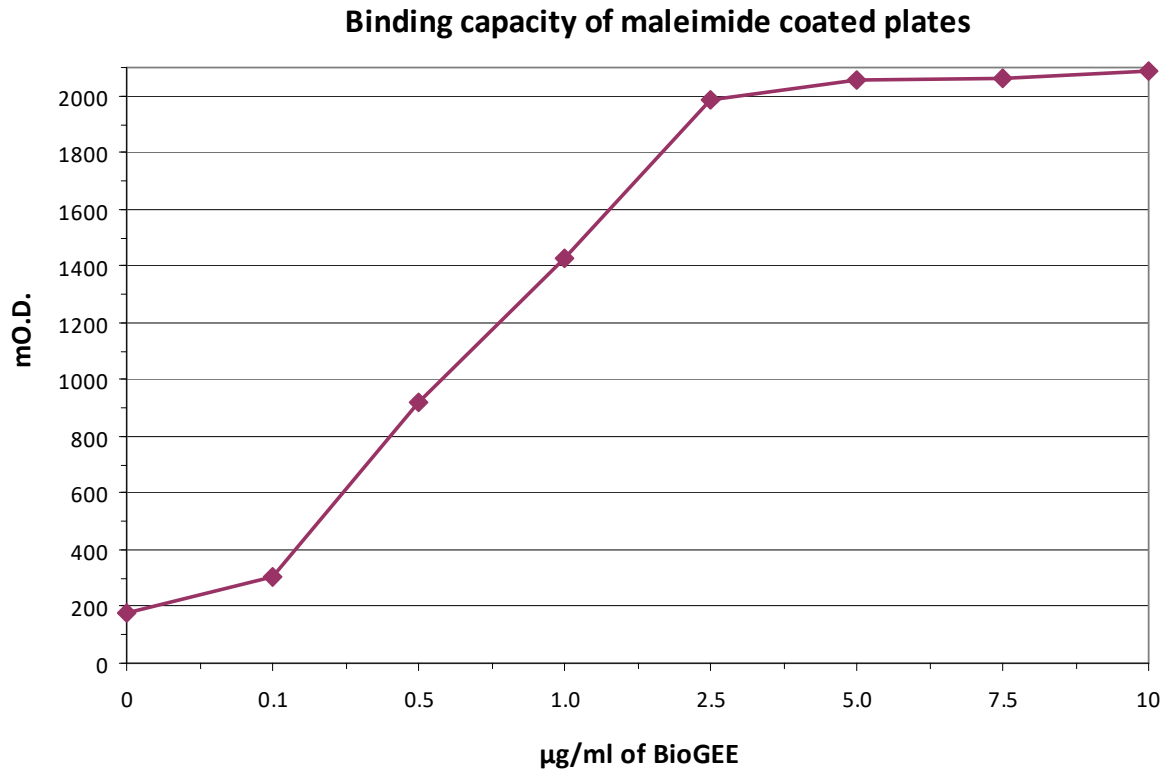
1. Add 100 µl of different concentrations of Glutathione Ethyl Ester Biotin Amide (BioGEE) from 0.1 to 10 µg/ml diluted in 10 mM PBS pH 6.6, 115 mM NaCl, 100 mM EDTA, 40 mM sucrose and incubate for 2 h at room temperature
2. Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween® 20 four times
3. Add 100 µl /well of Streptavidin-HRP (BioSpa product code SB01-61 at 1 mg/ml), diluted 1:5.000 and incubate for 45 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 10-15 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 a BioGEE concentration of 2.5µg/ml (figure 1).

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

- µg/well = 0.25 µg (250 ng/well)
- pMol/well= 440 (this result is calculated considering the BioGEE M.W. = 561 Da)

Figure 1



NEUTRAVIDIN®

The neutravidin coated surfaces offer a powerful and universal instrument for binding biotinylated molecules minimizing non-specific interactions.
Neutravidin is a deglycosylated avidin (M.W. 60 kDa) that contains four identical subunits biotin-binding with very high affinity for biotin (Ka= 10^{-15} M).
It has an isoelectric point near-neutral (pI=6,3) and the lowest non specific binding properties among the known biotin binding proteins.
Compared to the streptavidin, neutravidin in its primary structure does not contain the RYD sequence (Arg-Tyr-Asp): the lack of such a sequence totally eliminates the possibility to interact with the RGD sequence (Arg-Gly-Asp) present in the membrane receptors of a large variety of cells.
This makes it very useful in applications where it is important to avoid the non-specific interaction with the cell surfaces.

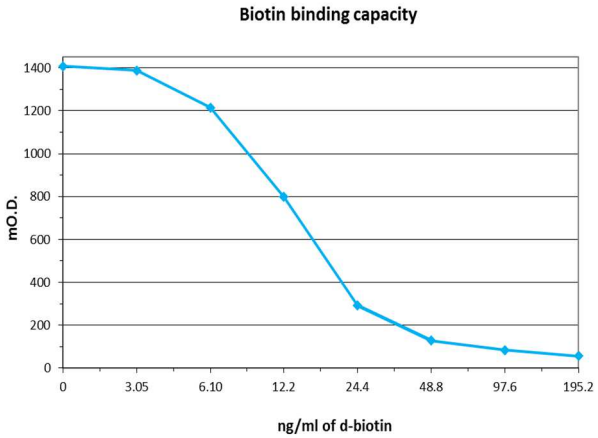
Neutravidin coated surface has similar binding capacity of **streptavidin coated surface** for biotin and biotinylated molecules.

TECHNICAL NOTE N. 32

Functional features of neutravidin coated plates

The following parameters were analysed
1. binding capacity towards biotin
2. binding capacity towards biotinylated IgG
3. uniformity
4. stability tests

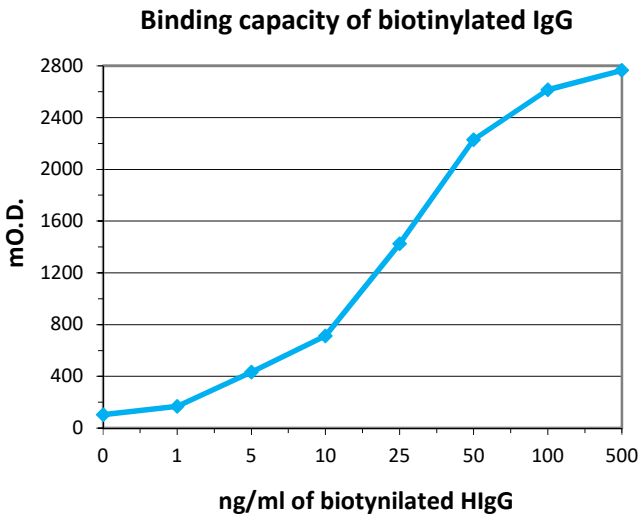
1. Binding capacity of a small molecule: biotin
Neutravidin coated wells were incubated with biotin solutions (from 0 to 195.2 ng/ml) containing 2 ng/ml of biotinylated peroxidase for 30 minutes at room temperature.
After a washing step, the wells were incubated with TMB and blocked with sulphuric acid 1N.
The OD values were read at 450 nm.



Results	1.22 ng*/well (100 µl volume) = ~ 5 pmol/ well (100 µl volume) 1.22 ng* d-biotin = 5 pmol

2. Binding capacity towards biotinylated HIgG

Neutravidin coated wells were incubated with solutions (from 0 to 500 ng/ml) of biotinylated HIgG for 30 minutes at room temperature.
After a washing step, the wells were incubated with AHIgG-HRP for 30 minutes at room temperature, again washed and incubated with TMB and blocked with sulphuric acid 1N.
The OD values were read at 450 nm.



Neutravidin coated wells are saturated from the concentration of 100 ng/ml biotinylated IgG.

3. Uniformity of biotin binding

Test conditions:

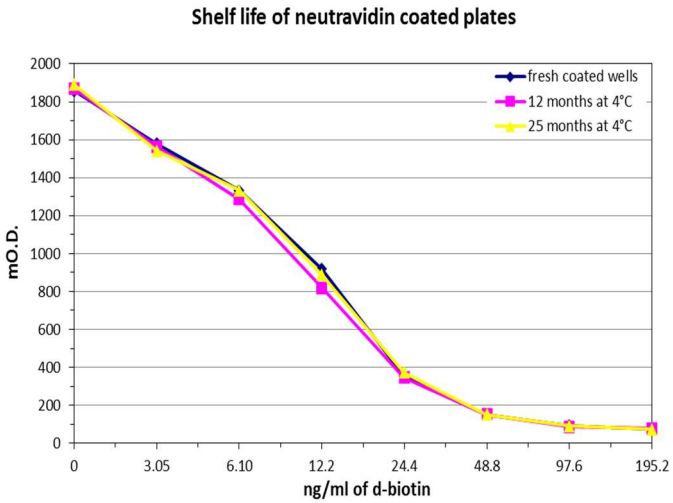
- A 96 wells plate was incubated with 2 ng/ml of biotinylated peroxidase
- After a washing step, the plate was incubated with TMB, then the reaction was stopped adding sulphuric acid 1N
- The optical density was determined at 450 nm and used for calculating the CV%

uniformity	CV% < 5

4. Stability tests

Neutravidin coated plates were maintained for 12 and 25 months stored at 4°C and tested in comparison with fresh samples.
Samples were incubated with biotin solutions (from 0 to 195.2 ng/ml) containing 2 ng/ml of biotinylated peroxidase for 30 minutes at room temperature.
After a washing step, the plates were incubated with TMB and blocked with sulphuric acid 1N.
The OD values were read at 450 nm.

The results show the stability of the coating.



POLY- L-LYSINE

Biomat has developed a polystyrene surface with physically adsorbed poly-L-Lysine. The monomeric L-Lysine chain shows a high density of groups:

- α-amino
- α-carboxyl
- ε-amino

These groups are able to react trough electrostatic and stereospecific bonds. The polystyrene optical features don't change, allowing the modified surface to be used as a valid tool to carry out biological tests.
This surface shows its usefulness for these applications:

- interactions with plasminogen and plasminogen activator
- interactions with ribosomal RNA
- interactions with double stranded DNA

TECHNICAL NOTE N. 21

Stereospecific binding activity

General procedure for binding NHS-b to Poly-L-Lysine coated surface

This test is suitable for measuring the available ε- amino groups on Lysine

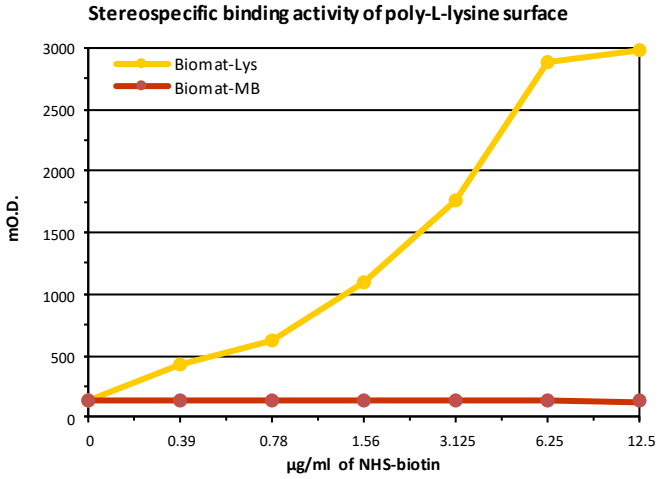
Preparation of reagents and buffers

Materials

Solid phase:	Biomat plates	MT12F-LYS-L (poly-L-Lysine coated plate) MT0F-MB (medium binding capacity)
ε-Caproylamido-biotin-N-hydroxysuccinimide ester (NHS- biotin)	BIO-SPA	Cat No. B002-61
Dimetilformamide (DMFO)	Fluka	Cat No. 40250
Tween® 20	Merck	Cat No. 822184
Streptavidin-peroxidase conjugate	BIO-SPA	Cat. No. SB01-61
TMB peroxidase substrate	Kirkegard & Perry	Cat. No. 50-76-05

Experiment

1. Dispense 100µl NHS-biotin solutions 12.5 – 6.25 – 3.125 – 1.56 – 0.78 - 0 µg/ml diluted in 0.1M PBS+ Tween® 20 0.15% pH 7.2 into the wells. Seal the wells with adhesive tape to prevent evaporation.
2. Incubate overnight at 4°C.
3. Empty the wells and wash with 0.1M PBS+ Tween® 20 0.05%, pH 7.2 four times.
4. Add 100µl of 50 ng/ml streptavidin-HRP to each well and incubate 30 minutes at room temperature.
5. Empty the wells and wash with 0.1M PBS+ Tween® 20 0.05%, pH 7.2 four times.
6. Add 100 µl /well of TMB substrate solution and incubate 10 minutes at room temperature.
7. Stop the substrate reaction by adding 100 µl of sulphuric acid 1 N and read the optical density values at 450 nm.



TECHNICAL NOTE N. 22

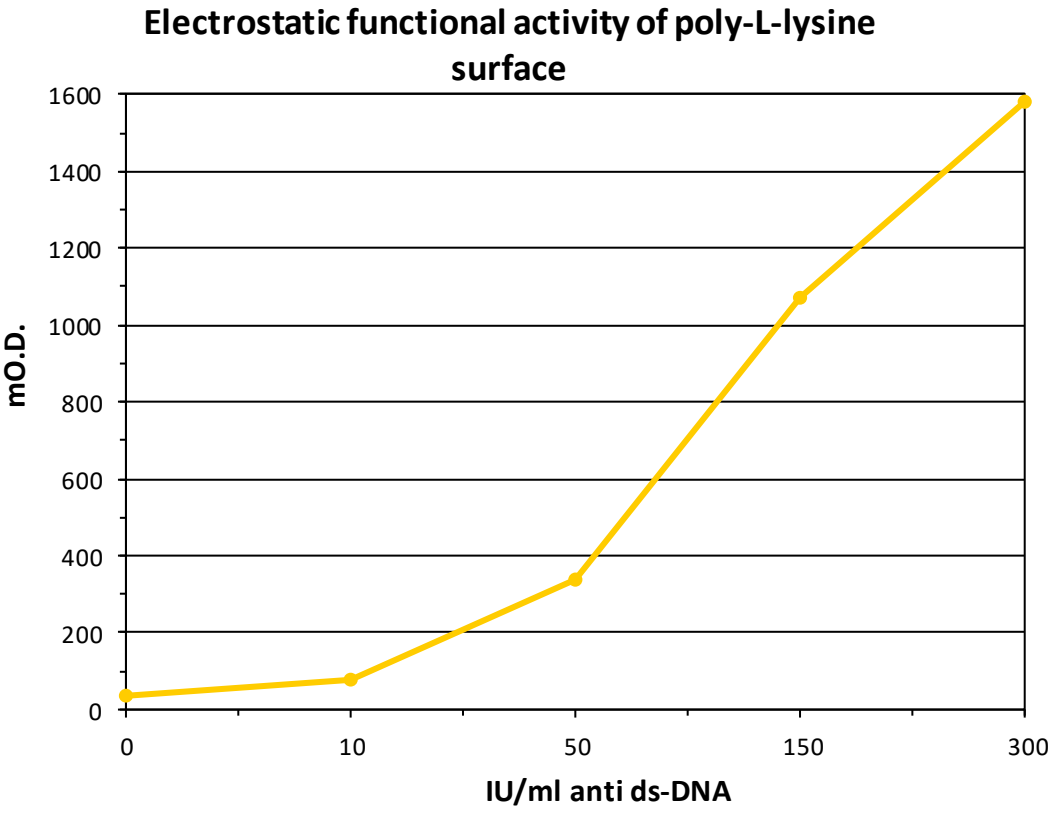
Electrostatic functional activity

General procedure for binding dsDNA to Poly-L-Lysine coated surface

1. dilute the dsDNA molecule to 1-10 μg/ml in 20 mM TRIS-HCl pH 8, 0.1 mM EDTA
2. proceed with incubation: conditions depend on dsDNA molecular weight and purity
3. wash three times to remove the unbound material
4. proceed with your specific test/application

example of test: human (autoantibodies) IgG determination to dsDNA

1. dilute dsDNA from *Calf tymus* (Sigma code D4522) to 5 μg/ml in 20 mM TRIS-HCl pH 8, 0.1 mM EDTA
2. add 100 μl/well of the diluted dsDNA to each wells and incubate o/n at + 4 °C
3. empty the wells and wash three times with 0.1 M PBS pH 7.2+0.05 % Tween® 20
4. add 200 μl to each wells of 0.1 M PBS pH 7.2, 0.5 % BSA and incubate 2 h at room temperature
5. empty the wells and wash three times with 0.1 M PBS pH 7.2+0.05 % Tween® 20
6. add 100 μl of diluted human serum with the following IgG concentrations to dsDNA:
7. 0-10-50-150-300 IU/ml
8. incubate 30' at room temperature
9. empty the wells and wash three times with 0.1 M PBS pH 7.2+0.05 % Tween® 20
10. add 100 μl of diluted goat anti-human IgG-peroxidase labeled
11. incubate 30' at room temperature
12. empty the wells and wash three times with 0.1 M PBS pH 7.2+0.05 % Tween® 20
13. add 100 μl/well of TMB substrate and incubate 15 minutes at room temperature
14. stop the substrate reaction by adding 100 μl of sulphuric acid 1N and read the optical density
15. values at 450 nm



POLY- L-ARGININE

Biomat has developed a polystyrene surface with physically adsorbed poly-L-Arginine. The monomeric L-Arginine chain shows a high density of groups:

- α-amino
- α-carboxyl
- guanidino

these groups are able to react trough electrostatic and stereospecific bonds.
The polystyrene optical features don't change, allowing the modified surface to be used as a valid tool to carry our biological tests.

This surface shows its usefulness for these applications:

- interactions with serino proteases
- interactions with maturation promoting factors

PROTEIN A

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 (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 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)

Coating

Recombinant Protein A (mol. weight 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 human IgG at a concentration of 7.5 µg/ml (750 ng/well) in an ELISA format using anti-human IgG-HRP as detector and TMB as substrate (see figure 1 for data and experiment details).
The Biomat Protein A microplate shows a nominal **binding capacity of ~ 5 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 A microplate shows a **sensitivity of 0.113 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, 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 NOTE N. 23

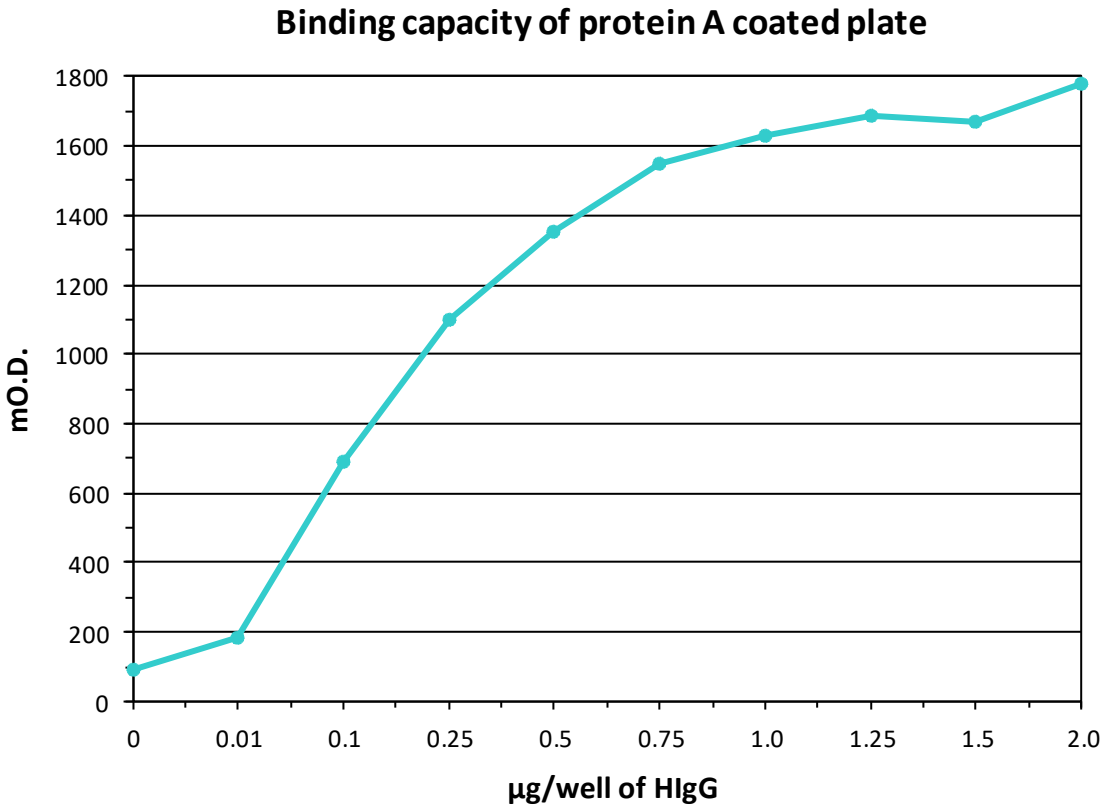
Binding capacity test

1. Add 100 µl of different concentrations of human IgG (from 0.1 to 20 µg/ml) to the wells of Protein A coated plate and incubate for 60 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 $F(ab)_2$ goat anti-human IgG-HRP (Sigma product code A 2290, diluted 1:8000) 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 7.5 µg/ml.
This concentration means the well binding capacity we can express as:

- µg/well = 0.75 (750 ng/well)
- pmol/well= 5 (this result is calculated considering the IgG M.W. = 150 kDa)

Figure 1



TECHNICAL NOTE N. 24

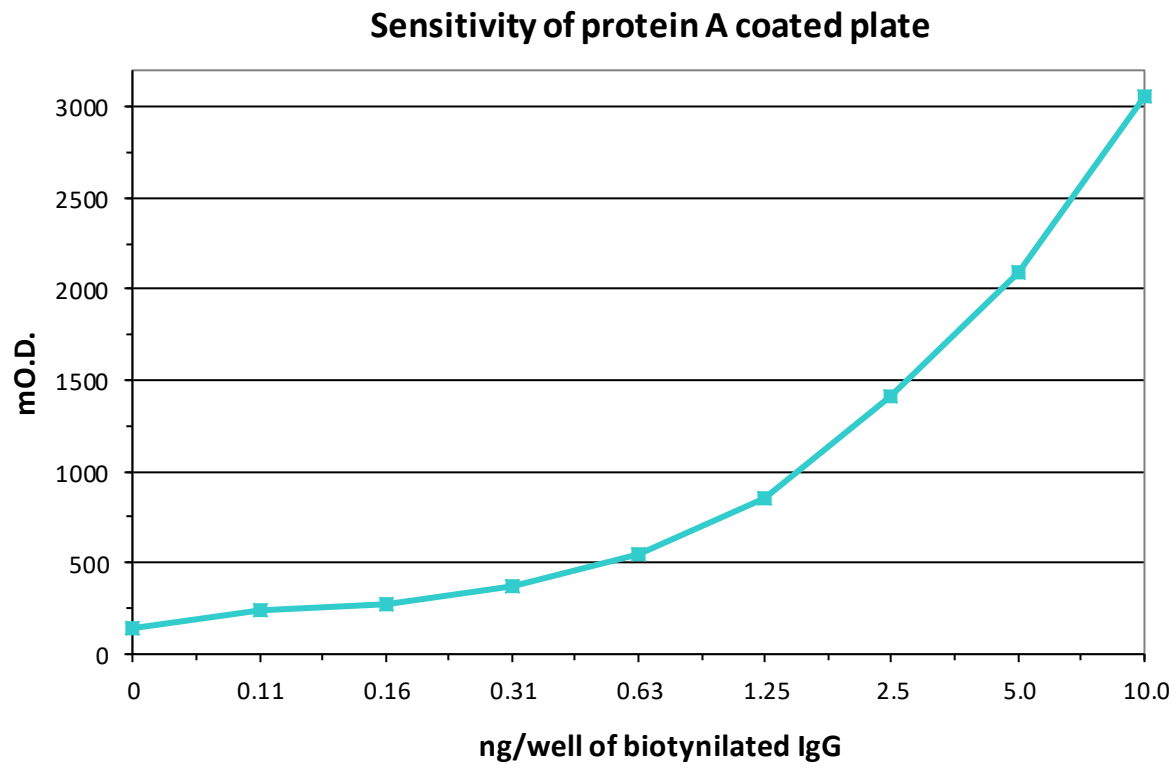
Sensitivity test

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® 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) = 0.141
- standard deviation = 0.019
- mean + 5 S.D. = 0.236
- sensitivity = 0.113 ng/well of human IgG

Figure 2



TECHNICAL NOTE 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.

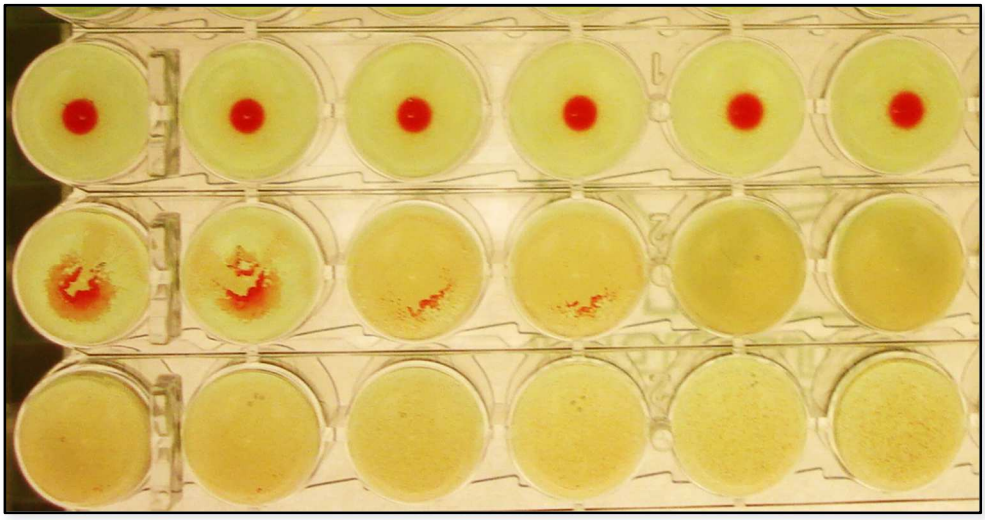
The indirect Coombs test is used in prenatal testing of pregnant women, and in testing blood prior to a blood transfusion. It detects antibodies against RBCs that are present unbound in the patient's serum. In this case, serum is extracted from the blood, and then the serum is incubated with RBCs of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



PROTEIN G

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 (see table 1 page 51), 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

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 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 desiccant and use within the expiration date.

TECHNICAL NOTE N. 26

Binding capacity test

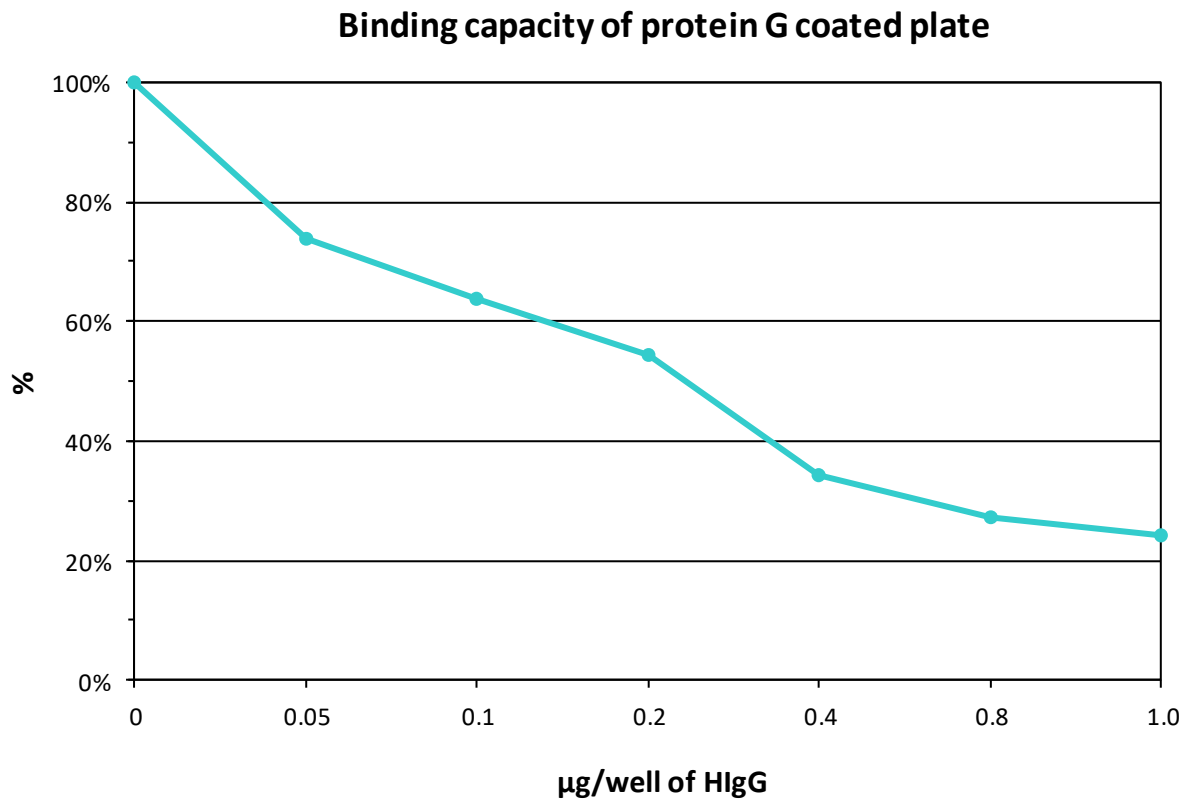
1. Add 100 µl of different concentrations of human IgG (from 0.5 to 10 µg/ml) mixed with a constant amount of human IgG biotinylated (0.01 µg/ml) to the wells of Protein G coated plate and incubate for 30 minutes at room temperature
2. Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween® 20 four times
3. Add 100 µl /well of Streptavidin-HRP (BioSpa product code SB01-61 at 1 mg/ml, 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 kDa)

Figure 1



TECHNICAL NOTE N. 27

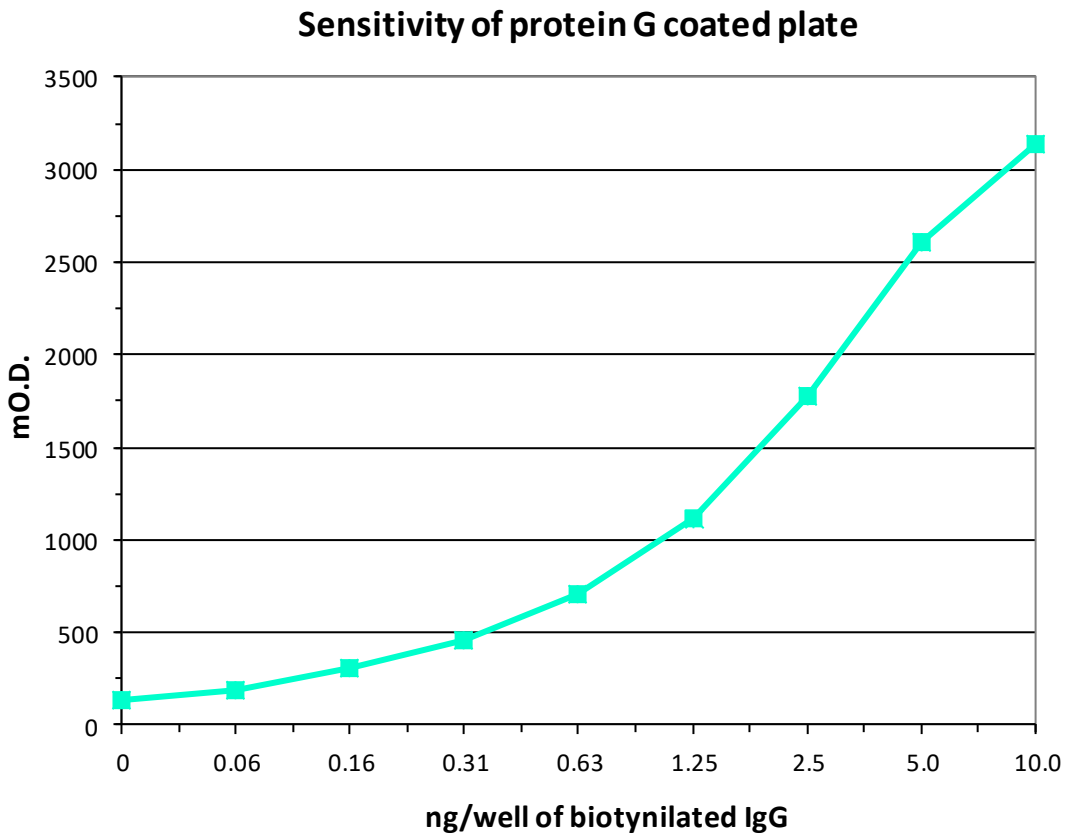
Sensitivity test

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® 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



PROTEIN A/G

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 versatile tool. The binding dependency to pH of Protein A/G is lower than Protein A but has the additive properties of Protein A and G together.
The Protein A/G binds to human IgG, IgA, IgM; it binds to all subclasses of mouse IgG excluding mouse IgA, IgM and serum albumin. When coated onto microplates, the Protein A/G can securely to be used in purification and detection of mouse monoclonal IgG antibodies with no interference from IgA, IgM and serum albumin.

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

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.

Uniformity

Microplates show a **CV% less than 5**.

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)

STREPTAVIDIN

Streptavidin coated surfaces offer a powerful and universal instrument for binding any biotinylated molecule (Proteins-Peptides-Polysaccharides-Oligonucleotides-DNA fragments-etc.)
Streptavidin is a tetrameric protein (M.W. 60 kDa) with very high affinity for biotin ($Ka=10^{-15}$ M); the bond is the strongest known non-covalent biological interaction.
Biotin is a small molecule which can be conjugated to many proteins without losing or altering their activity, each protein can bind many biotin molecules.
Since each subunit of streptavidin binds one molecule of biotin, the resulting effect is a great increase of the sensitivity of the assay.

The streptavidin-biotin bonding main features

- stability
- specificity
- affinity

make it useful for special applications of molecules which do not offer reliable bonding by passive adsorption or adsorb in an unfavourable orientation.

adsorb in an unfavorable orientation.

TECHNICAL NOTE N. 7

Functional features of streptavidin coated plates

The following parameters were analysed:
1. binding capacity towards biotin
2. specificity towards biotin
3. binding capacity towards biotinylated IgG
4. uniformity
5. stability tests:
   - 5.1 endurance under strong chemical contacts
   - 5.2 shelf life at 37°C
   - 5.3 temperature stress (transport simulation)
   - 5.4 long storage

1. Binding capacity towards biotin

Streptavidin coated wells (and BSA saturated control wells) were incubated with a calibrated biotin solution.

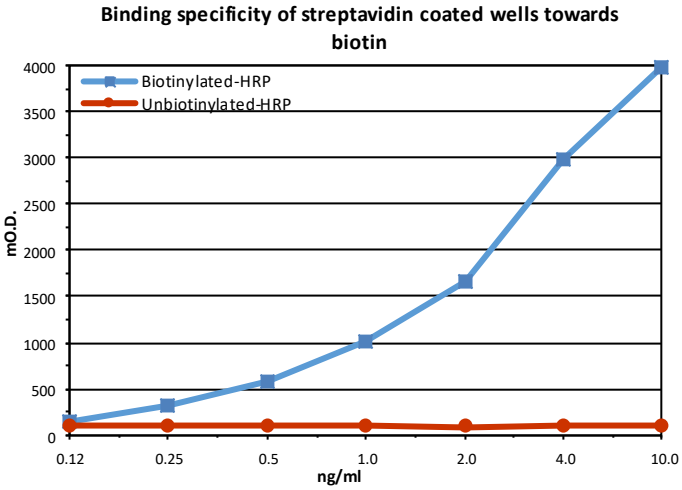
Subsequently, aliquots of this solution, concomitantly with biotin standards, were mixed with biotinylated peroxidase and transferred into new empty streptavidin coated wells. From the amount of enzyme bound to the solid phase, the biotin content of the samples was calculated.

This value was compared with the amount of biotin originally added; from the difference (corrected for-non specific binding of biotin to the control wells), the capacity of the wells for biotin was derived.

Results	12 pmol/ well (200 µl volume)

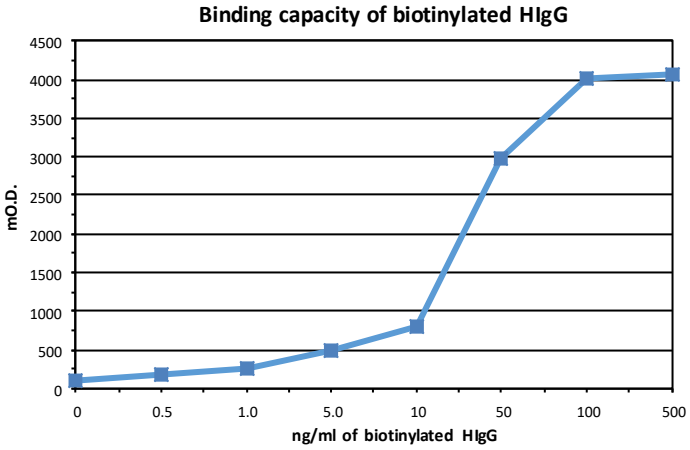
2. Specificity towards biotin

Streptavidin coated wells were incubated with solutions (from 10 to 0.12 ng/ml) of biotinylated peroxidase and unbiotinylated peroxidase (blanks) for 30 minutes at room temperature.
After a washing step, the wells were incubated with TMB and blocked with sulphuric acid 1N.
The OD values were read at 450 nm.



3. Binding capacity towards biotinylated HIgG

Streptavidin coated wells were incubated with solutions (from 0 to 500 ng/ml) of biotinylated HIgG for 30 minutes at room temperature.
After a washing step, the wells were incubated with AHIgG-HRP for 30 minutes at room temperature, again washed and incubated with TMB and blocked with sulphuric acid 1N.
The OD values were read at 450 nm.



Results	100 ng/ well (100 µl volume)

4. Uniformity of biotin binding

Test conditions:

- A 96 wells plate was incubated with 2 ng/ml of biotinylated peroxidase
- After a washing step, the plate was incubated with the TMB, then the reaction was stopped adding sulphuric acid 1N
- The optical density was determined at 450 nm and used for calculating the CV%

Uniformity	CV% < 5

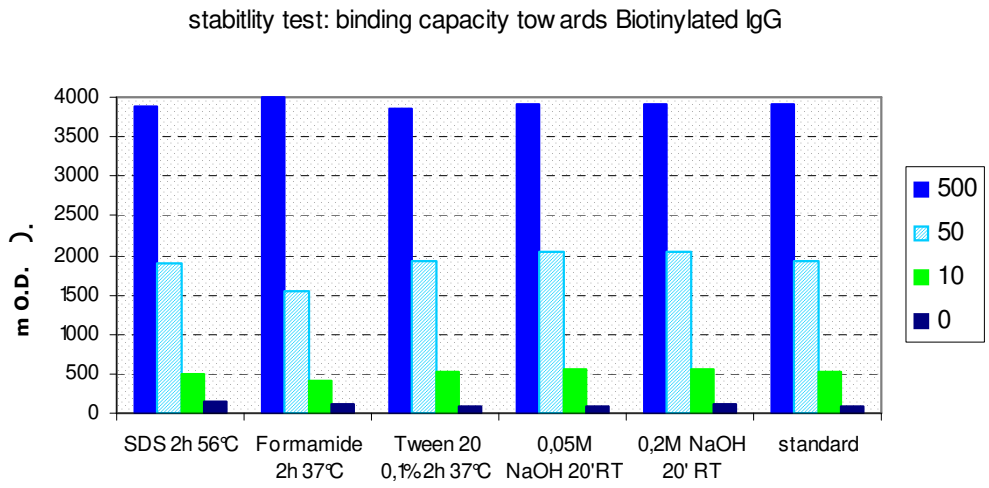
5. Stability tests:

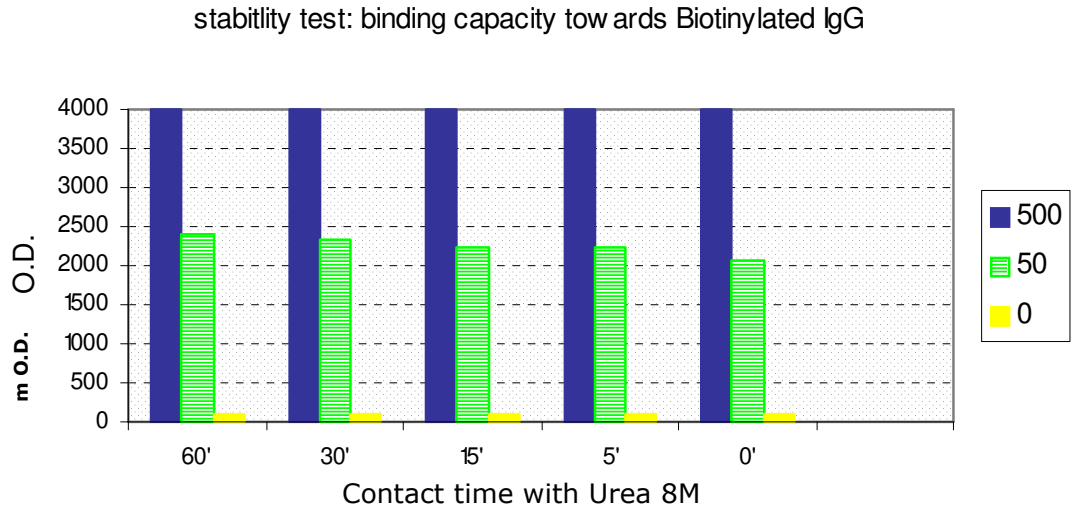
5.1. Endurance under strong chemical contacts

Endurance under strong chemical contacts was determined following method 1 (Biotinylated IgG from 500 to 0 ng/ml) where the first washing step was substituted by the following incubations:

chemical compound	conditions
SDS 0.1%, 0.6M NaCl	56°C 2h
30% Formamide in 0.6M NaCl	37°C 2h
0.1% Tween® 20 in 0.1M PBS	37°C 2h
0.05M NaOH	R.T. 20'
0.2M NaOH	R.T. 20'
standard - 0.1% Tween® 20 in 0.1M PBS	4 x
Urea 8M	60'-30'-15'-5'

Results





5.2. Shelf life at 37°C

Streptavidin coated wells maintained for 15 days at 37°C in comparison with standard stored at 4°C, were analysed with method 1 (biotinylated IgG =100 ng/ml)

temperature	4 ° C	37°C
m O.D.	2568	2667
CV%	3.3	3.6

5.3. Long storage

Streptavidin coated wells maintained for 30 months in a warehouse without air conditioning (temperature range from 10°C to 40 °C) in comparison with samples stored at 4°C (standard condition), were analysed with method 2.

temperature	4 ° C	R.T.
m O.D.	1890	1950
CV%	1.8	2.3

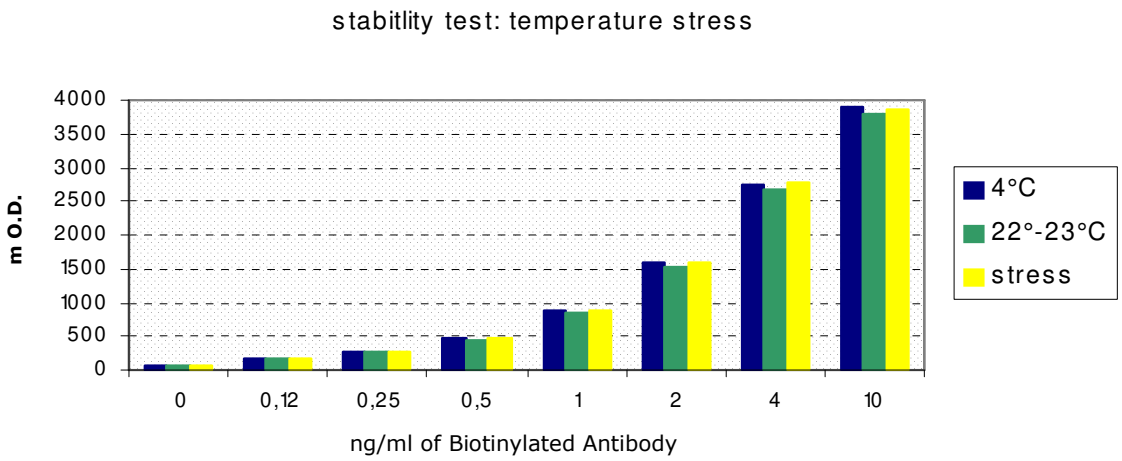
The results show the exceptional stability of the coating.

5.4. Temperature stress (transport simulation)

Stability was checked under different temperatures as those which may occur during transport.
Method 2 was used for comparing streptavidin coated plates subjected to the following conditions:

plate n.	conditions	time
1	4° C	10 days
2	22°-23° C	10 days
3	37° C 22°-23° C - 20° C 37° C 22°-23° C	3 days 12 hours 10 hours 3 days 3 days

Results



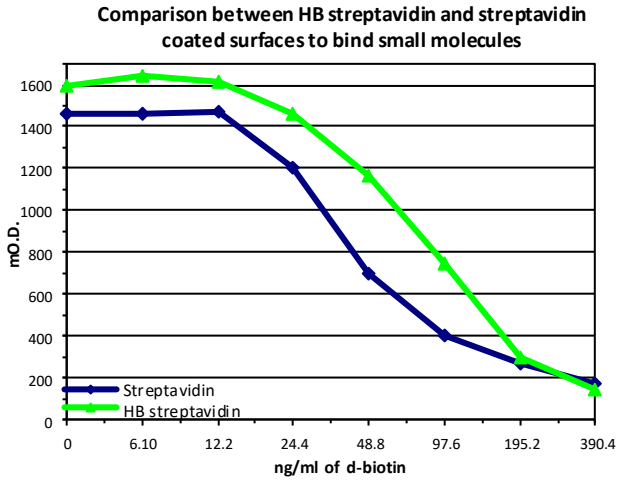
HIGH BINDING STREPTAVIDIN

HB streptavidin coated plate is a powerful and universal instrument for binding any biotinylated molecule (Proteins-Peptides-Polysaccharides-Oligonucleotides-DNA fragments-etc.).
Biotin is a small molecule which can be conjugated to many proteins without losing or altering their activity, each protein can bind many biotin molecules.
Since each subunit of streptavidin binds one molecule of biotin, the resulting effect is a great increase of the sensitivity of the assay.
Unlike the normal Streptavidin coated, these plates are particularly useful in competitive tests to measure biotinylated low molecular weight molecules.

TECHNICAL NOTE N. 31

1. comparison between HB streptavidin and streptavidin coated surfaces to bind small molecules (biotin, M.W. = 244 Da)

HB streptavidin and streptavidin coated wells were incubated with biotin solutions (from 0 to 390.4 ng/ml) containing 2 ng/ml of biotinylated peroxidase for 30 minutes at room temperature.
After a washing step, the wells were incubated with TMB and blocked with sulphuric acid 1N.
The O.D. values were read at 450 nm.



The two microplates show different binding curves towards biotin.

Streptavidin coated plate shows a binding capacity of ~ 2.2 ng*/ well (100 µl volume)
*2.2 ng d-biotin = 9 pmol

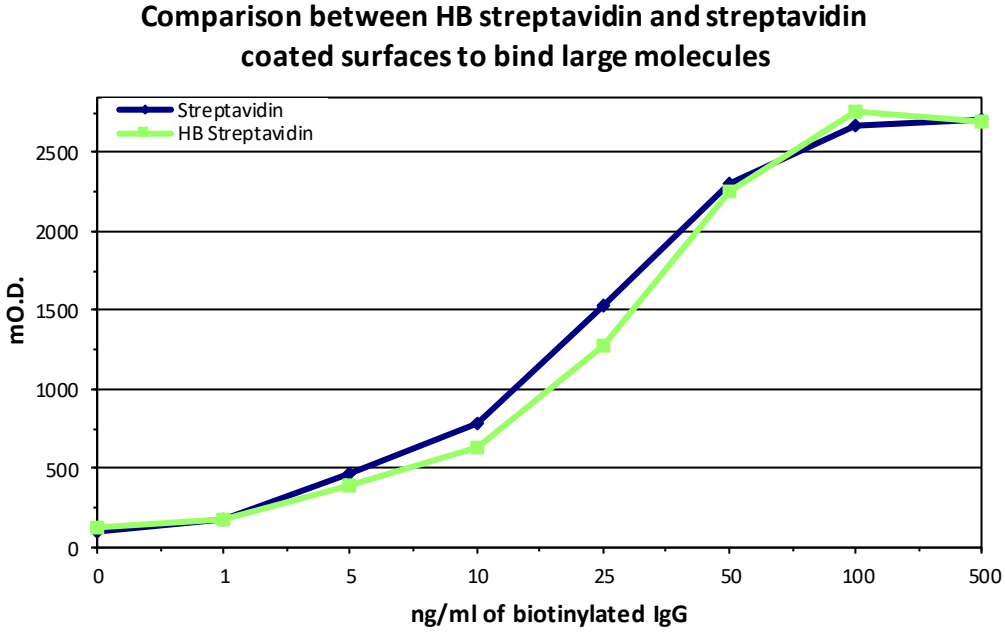
HB streptavidin coated plate shows a binding capacity of ~ 9 ng**/ well (100 µl volume)
** 9 ng d-biotin = 37 pmol

Binding capacity of HB streptavidin coated plate	> 35 pmol/ well (100 µl volume)

2. comparison between HB streptavidin and streptavidin coated surfaces to bind large molecules (IgG, M.W. = 150 kDa)

HB streptavidin coated wells were incubated with solutions (from 0 to 500 ng/ml) of biotinylated HIgG for 30 at room temperature.
After a washing step, the wells were incubated with AHIgG-HRP for 30 at room temperature, again washed and incubated with TMB and blocked with sulphuric acid 1N.
The OD values were read at 450 nm.

The two microplates show the same bond curve towards biotinylated HIgG and they are saturated from the concentration of 100 ng/ml biotinylated HIgG.



3. Uniformity of biotin binding

Test conditions:

- A 96 wells plate was incubated with 2 ng/ml of biotinylated peroxidase
- After a washing step, the plate was incubated with the TMB, then the reaction was stopped adding sulphuric acid 1N
- The optical density was determined at 450 nm and used for calculating the CV%

Uniformity	CV% < 5

WHEAT GERM

Wheat Germ Lectin, belonging to the lectins family, is a Hemagglutinin obtained from the common wheat germ *Triticum Vulgaris*. It is well known that lectins have been used extensively for the isolation of glyco-conjugates and glycoproteins with specific carbohydrate structures.
Wheat Germ Lectin shows specific affinity for molecules containing N-acetyl-D-glucosamine residue.
Wheat Germ Lectin coated surfaces offer a powerful and sensitive instrument for binding in specific way the carbohydrate fraction of glycoproteins, enzymes and cell membranes.
The optical properties of polystyrene remain unchanged, allowing to use the modified surface as powerful tool for diagnostic assays.

Example of applications:

- studies of surfaces of normal and transformed cells
- glycoprotein purification including membrane glycoproteins
- studies of cell surface changes during development and the cell cycle

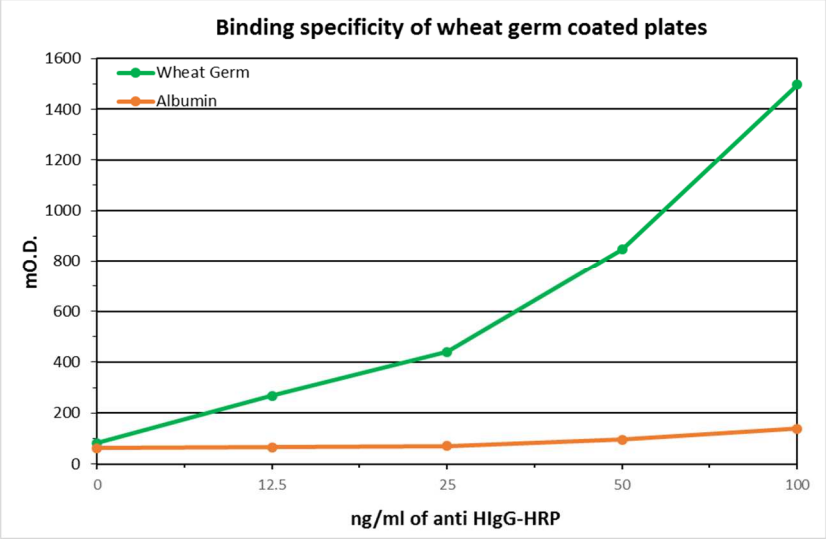
TECHNICAL NOTE N. 17

General procedure for binding a biomolecule to Wheat Germ coated surface

1. Dilute your biomolecule (sample) to 0.5- 5 μg/ml in an appropriate neutral pH buffer (buffer should contain 1mM Ca^{++} and 1mM Mn^{++}; in fact these ions promote the interaction between saccharide groups and Wheat Germ coated surface)
2. Proceed with incubation: conditions depend on biomolecule structure
3. Wash four times to remove the unbound material
4. Proceed with your specific test: to point out the bound biomolecule and/or to use the bound biomolecule to point out a specific counter molecule

Example of test: binding specificity of Wheat Germ coated plates

1. Dilute aHIgG-HRP from 100 ng/ml to 12.5 ng/ml in pure distilled water containing 1 mM $CaCl_2$•2 H_2O + 1 mM $MnCl_2$•4 H_2O
2. Add 100μl of each solution to the wells of Wheat Germ coated plate and incubate 30 minutes at room temperature; add the same solutions to albumin coated plate as comparison for evaluate the specificity of binding
3. Leave blank wells as control
4. Empty the wells and wash with 0.1M PBS pH 7.2 + 0.05% Tween® 20 four times
5. Add 100 μl /well of TMB substrate solution and incubate 10 minutes at room temperature
6. Stop the substrate reaction by adding 100 μl of sulphuric acid 1 N and read the optical density at 450 nm



AMINATED SURFACE

Surfaces with primary amino groups covalently bound are dedicated to promote the covalent immobilization of compounds containing reactive moieties such as amino, carboxyl or thiol groups via well-known homo-heterobifunctional linkers, e.g. N-Hydroxysuccinimide (NHS) or Succinimidyl 4-(N-maleidomethyl) cyclohexane-1-carboxylate (SMCC).

This kind of immobilization can overcome some of the limitations connected with physical adsorption of the molecules to the surfaces such as:

- immobilization of molecules which are bound weakly or not at all by physical adsorption, namely small peptides (M.W. 1000-5000 Da) drugs, toxins or hormones
- oriented immobilization of molecules in order to secure the integrity and accessibility of their specific sites avoiding the risk of inhibition of these sites by casual physical adsorption for such molecules as Fab-SH-antibody fragments, streptavidin, polysaccharides or nucleic acids (single strand or double strand)

- increased storage stability compared with that of physical adsorption because of the reduced risk of spontaneous desorption

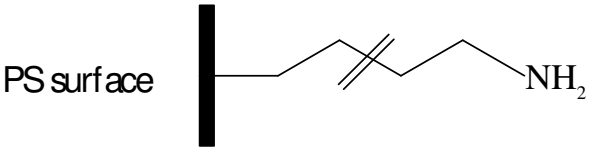
TECHNICAL NOTE N. 6

Introduction to the preparation and use of aminated surfaces for immunological assays

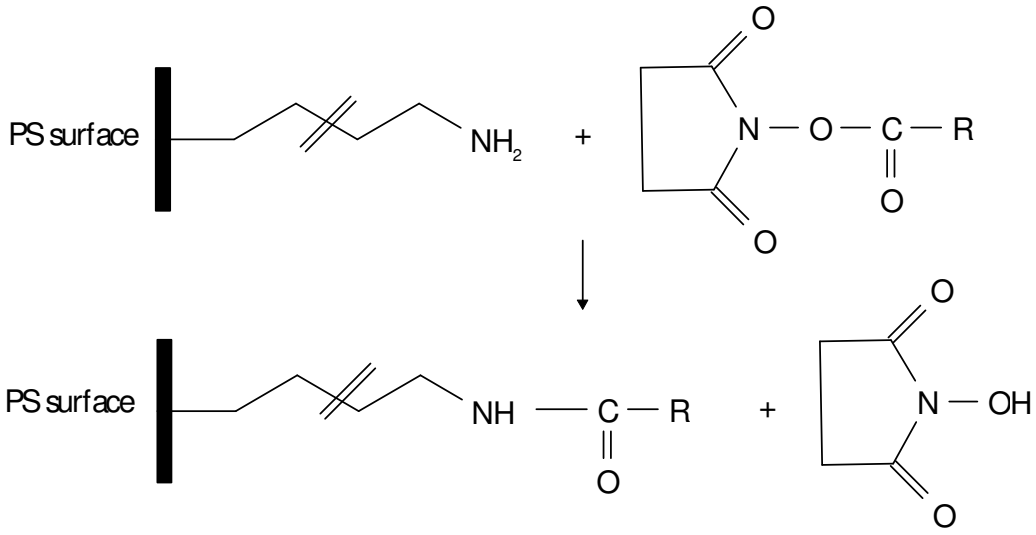
Direction for use

Several recipes are routinely used for the coupling of biological molecules to amino groups. Specific directions for use require the knowledge of the intended application. As general guideline, the interaction between the amino group on the surface and the functional group of the molecule to be bound is based on covalent binding mediated by homo and heterofunctional crosslinkers.
In particular, Ethyldiethylaminopropylcarbodiimide (EDC), with or without the addition of N-hydroxysuccinimide, is a powerful coupling agent of the carboxylic group of the molecule with the amino group of the surface.
If the biomolecule to be bound contains ε amino groups of lysine, the simplest method is coupling via Glutaraldehyde, with the formation of a stable amine linkage by reduction with Sodium Cyanoborohydride.
Other crosslinkers for this purpose are Dimethylpimelidate and Dissucinimidyl suberate.
Biomolecules containing thiolic groups, as Fab-SH or peptides with cystein at terminal end, can exploit the large number of maleimido groups containing crosslinkers as Succinimidyl 4-(N-maleimidomethyl)cycloexane-1-carboxylate (SMCC) for reacting with the amino group.

Schematic chemical and physical configuration of Biomat NH_2 surface



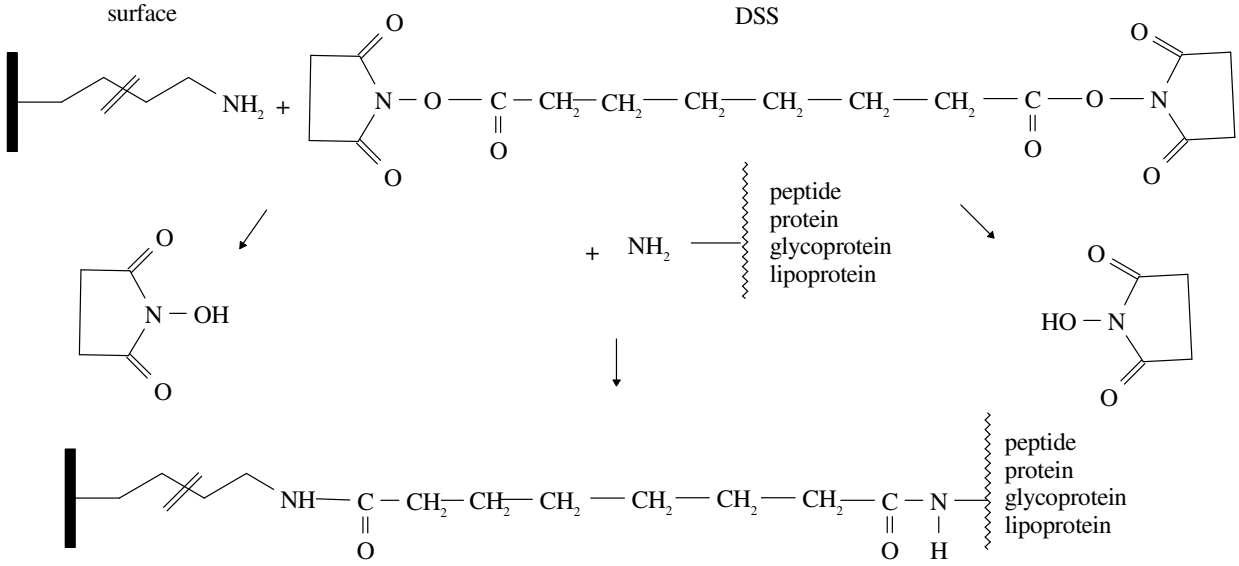
example of reaction scheme:
an arbitrary NHS estherified compound (R) covalently combines with Biomat NH_2 surface through NHS splitting off



Hereunder are some examples of coupling agents to be used for covalent coating of the Biomat NH_2 surface with reactive groups

A. Disuccinimidyl suberate (DSS).
This symmetric (homobifunctional) linker is capable of linking compounds containing secondary or primary amino groups, and can thus be used for covalent immobilisation of peptides, proteins, glycoproteins, lipoproteins

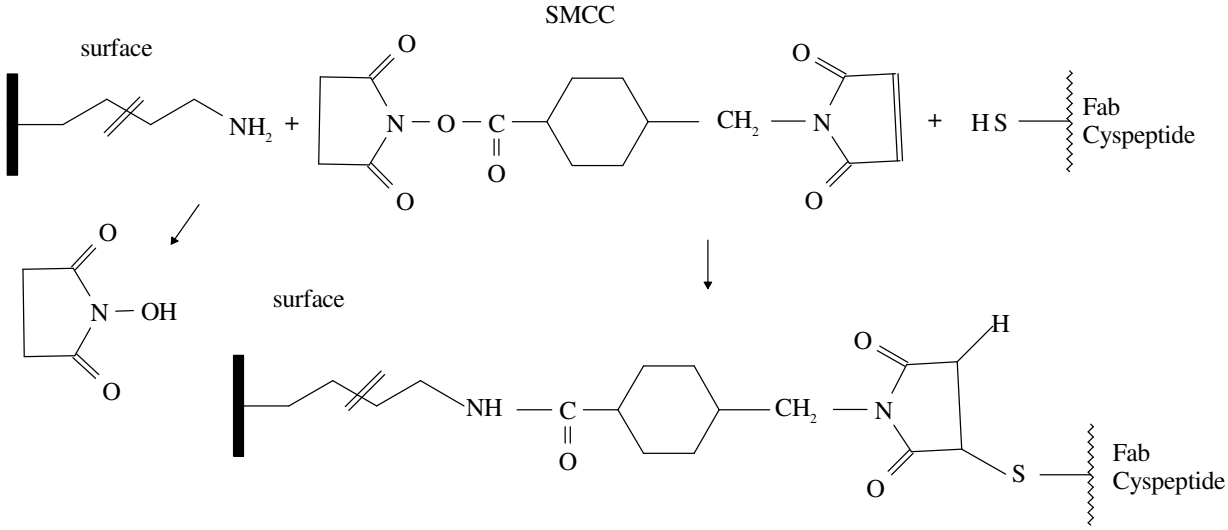
Reaction A



B. Sulfosuccinimidyl maleimidomethyl cyclohexane carboxylate (SMCC).

This heterobifunctional linker is capable of linking compounds with SH-containing compounds. It can be used especially for covalent immobilisation of Fab-SH-antibody fragments or terminally cysteinized antigenic peptides, thereby exposing the active ends of these compounds to the liquid phase.

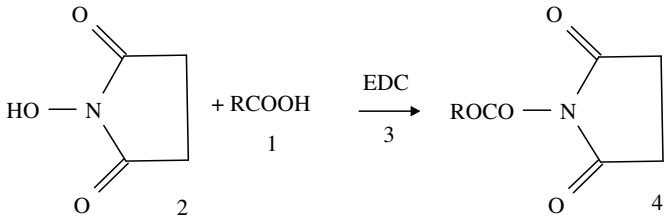
Reaction B

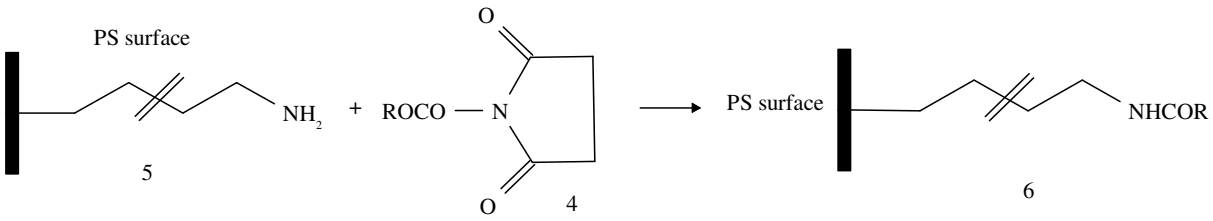


C. N-Hydroxysulfosuccinimide (Sulfo-NHS) or N-Hydroxysuccinimide (NHS) combined with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide Hydrochloride (EDC).

The EDC linker combined with sulfo-NHS is capable of linking small peptides (M.W. around 1000) via their carboxyl group to the NH-activated strips surfaces.

Reaction C





1. Peptide
2. Sulfo NHS (or NHS)
3. EDC
4. Intermediate active compound resulting from the reaction
5. Biomat NH_2 surface
6. Peptide covalently immobilised on Biomat NH_2 surface

TECHNICAL NOTE N. 12

General directions for the use of surfaces with amino and carboxylic groups

Biomat has developed modified polystyrene surfaces introducing chemical groups such as NH_2 and COOH. These groups are able to covalently bind compounds to the plastic surface. The optical properties of polystyrene remain unchanged, allowing to use the modified surfaces as powerful tools for diagnostic assays.

These surfaces offer the possibility to

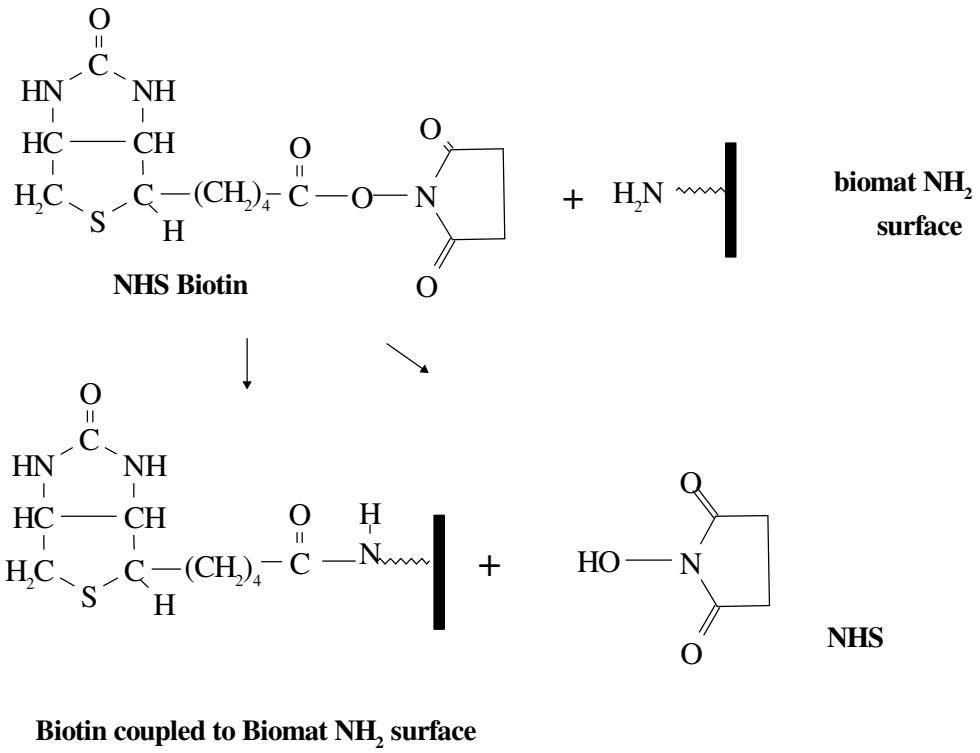
- covalently immobilize small molecules which only bind weakly or not at all by physical adsorption
- orientate the immobilization of molecules in a defined way on the solid phase.

Hereunder are some examples of application that can be used as guidelines to enable users to develop their own bio-specific assays.

1. Coupling of NHS-activated compounds

A very simple and easy application of Biomat NH_2 surfaces is coupling of molecules that have been activated by esterification with N-hydroxysuccinimide derivatives (NHS). In our experiment an-Hydroxysuccinimide active ester of biotin links immediately via its carbonilic group to the surface amino groups as shown in figure 1.

Figure 1



Preparation of reagents and buffers

Materials

Solid phase:	Biomat plates	MT02F2-AM1 (primary amino groups) MG01F-HB (high binding capacity)
ε-Caproylamido-biotin-N-hydroxysuccinimide ester (NHS- biotin)	BIO-SPA	Cat No. B002-61
Dimetilformamide (DMFO)	Fluka	Cat No. 40250
Tween® 20	Merck	Cat No. 822184
Streptavidin	BIO-SPA	Cat. No. S002-60
Streptavidin-peroxidase conjugate	BIO-SPA	Cat. No. SB01-61
BSA	Intergen	Cat. No. 3100
TMB peroxidase substrate	Kirkegard & Perry	Cat. No. 50-76-05

NHS-Biotin stock solution

NHS-biotin	6 mg
DMFO	2 ml

NHS-Biotin solution 150 µg/ml

NHS Biotin stock solution 500 µl

PBS 0.1M pH 7.2+0.15% Tween® 20 →10 ml

NHS-Biotin solution 100 µg/ml

NHS Biotin stock solution 333 µl

PBS 0.1M pH 7.2+0.15% Tween® 20 →10 ml

NHS-Biotin solution 50 µg/ml

NHS Biotin stock solution 167 µl

PBS 0.1M pH 7.2+0.15% Tween® 20 →10 ml

NHS-Biotin solution 10 µg/ml

NHS Biotin stock solution 33 µl

PBS 0.1M pH 7.2+0.15% Tween® 20 →10 ml

Streptavidin-mix

Streptavidin	50 µg
Streptavidin-peroxidase	1 µg
PBS-BSA 1%	10 ml

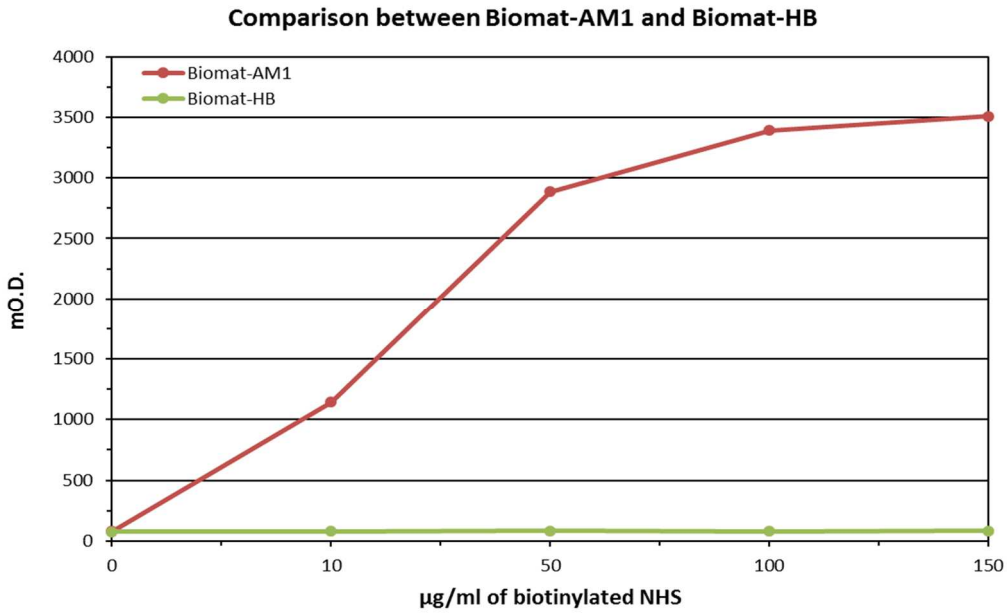
Experiment

1. Add 100 µl NHS-biotin solutions 150-100-50-10 µg/ml and 0.1M PBS + Tween® 20 0.15% pH 7.2 as 0 µg/ml to the wells (with primary amines and HB). Seal the wells with adhesive tape to prevent evaporation
2. Incubate overnight at room temperature
3. Empty the wells and wash with 0.1M PBS + Tween® 20 0.05%, pH 7.2 four times
4. Add 100 µl of streptavidin mix to each well and incubate 30 minutes at room temperature
5. Empty the wells and wash with 0.1M PBS + Tween® 20 0.05%, pH 7.2 four times
6. Add 100 µl/well of TMB substrate solution and incubate 10 minutes at room temperature
7. Stop the substrate reaction by adding 100 µl of sulphuric acid 1 N and read the optical density values at 450 nm

Results

The results (see figure 2) show a clear correlation between the concentration of NHS-biotin added to the wells and the amount of biotin bound to the Biomat NH_2 surface.
On the other side no biotin is bound onto the plate without primary amino groups grafted to its surface, showing that passive adsorption of neither biotin nor enzyme conjugate occurs.
We therefore conclude that NHS-Biotin has indeed been covalently bound to the amino groups present on the Biomat NH_2 surface.

Figure 2

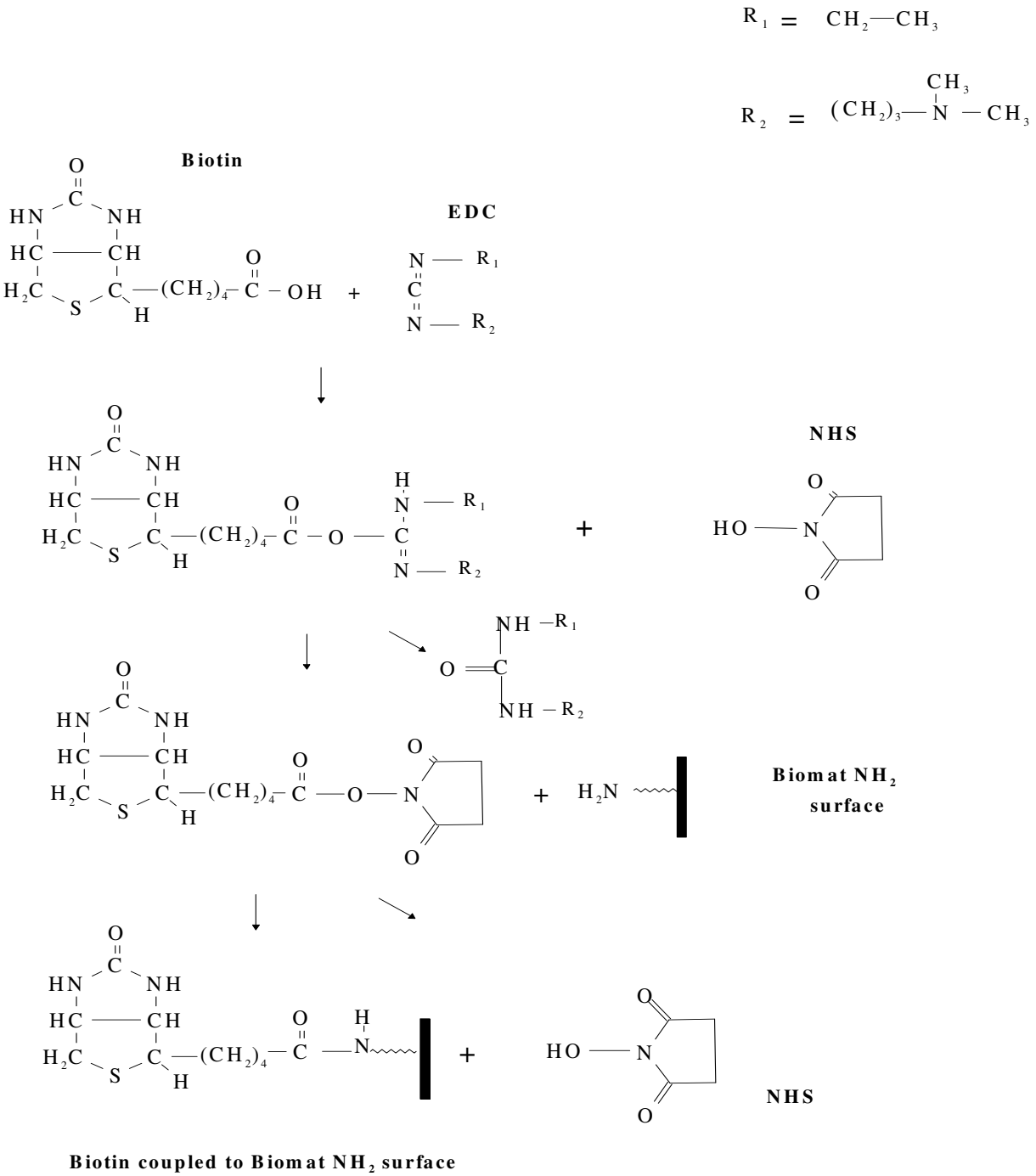


2. Coupling hapten or peptide, having a carboxylic group, to Biomat NH_2 surface

The carboxylic group presents in a molecule with a low molecular weight, such as a hapten or a peptide, binds to Biomat NH_2 through formation of amide bonds between the carboxylic group presents in the molecule and the surface amino group by the combined action of carbodiimide and N-hydroxysuccinimide.

The figure 3 shows the reaction scheme for coupling of the hapten, biotin, through its available carboxylic group.

Figure 3



Preparation of reagents and buffers

Materials

Solid phase:	Biomat plates	MG02F-AM1(primary amino groups) MG01F-HB (high binding capacity)
d-Biotin	Sigma	Cat. No. B 4501
1-Ethyl-3-(3 dimethylaminopropyl)-carbodiimide (EDC)	Sigma	Cat. No. E 1769
Sulfo-N-hydroxysuccinimide (sulfo-NHS)	Fluka	Cat. No. 56485
Dimethylsulfoxide (DMSO)	Merck	Cat. No. 2931
Tween® 20	Merck	Cat. No. 822184
Streptavidin	BIO-SPA	Cat. No. S002-60
Streptavidin-peroxidase conjugate	BIO-SPA	Cat. No. SB01-61
BSA	Intergen	Cat. No. 3100
TMB peroxidase substrate	Kirkegard & Perry	Cat. No. 50-76-05

Biotin stock solution

d-Biotin	7.8 mg
DMSO	0.5 ml
Distilled water	0.5 ml

EDC solution

EDC	5.8 mg
Distilled water	to 10 ml

Biotin/NHS solution

Biotin stock solution	500 µl
Sulfo-NHS	3.45 mg
Distilled water+0,30% Tween® 20	to 10 ml

Streptavidin-mix

Streptavidin	50 µg
Streptavidin-peroxidase	1 µg
PBS-BSA 1%	10 ml

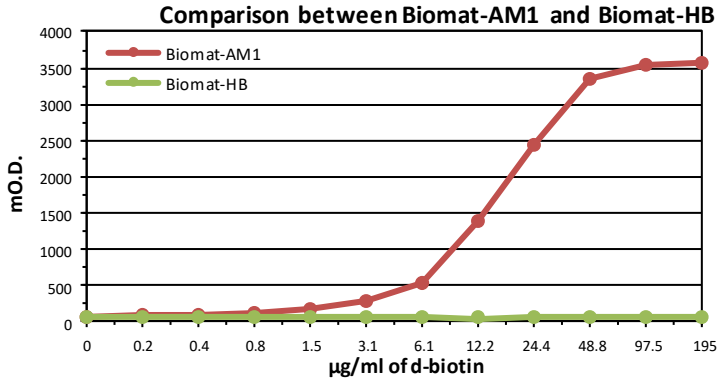
Experiment

1. Add 50 µl of distilled water to each well, apart from wells in column 2. Then add 100 µl of Biotin-NHS solution to all wells in column 2
2. Dilute by transferring 50 µl from the wells in column 2 to column 3, mix, transfer 50 µl from column 3 to column 4, mix and proceed in this way up to column 12
3. To start reaction: add 50 µl of EDC solution to each column. In blank experiment (column 1) add 50 µl of distilled water instead of EDC
4. Incubate at room temperature for 2 hours
5. Empty the wells and wash with 0.1M PBS+0.05% Tween® 20 pH 7.2 four times
6. Add 100µl of streptavidin mix to each well and incubate for 30 minutes at room temperature
7. Empty the wells and wash with 0.1M PBS+0.05% Tween® 20 pH 7.2 four times
8. Add 100 µl of TMB substrate solution to each well and incubate for 10 minutes at room temperature
9. Stop the substrate reaction by adding 100 µl of sulphuric acid 1 N and read the optical density values at 450 nm

Results

The results of this experiment (figure 4) clearly show that the molecule (biotin) is bound in a detectable way to the Biomat NH_2 (cod. AM1) whereas no detection could be obtained on Biomat HB.
The results indicate that a covalent coupling has taken place between the carboxylic group in the biotin and the primary amino group grafted on the Biomat NH_2. The results (data not displayed) point out that without adding carbodiimide the covalent binding of biotin does not occur.

Figure 4



Examples of application

TECHNICAL NOTE N. 8

1. DNA binding on secondary amine support

Phosphorylation and labeling of the capture probe

DNA used for carbodiimide-mediated binding is phosphorylated at their 5' end with T_4 polynucleotide kinase and an aliquot is radiolabeled with [γ-^{32}P]-ATP by chromatography on Sephacryl 200 spin column (1 ml) (Pharmacia, Uppsala, Sweden). DNA concentration is then measured by fluorescence using the Hoechst H33258 compound (Labarca and Paigen,1980).
The purity of the fragment can be checked by agarose gel electrophoresis. Radiolabeled DNA is mixed with cold DNA in the ratio of 1:10 in order to reduce the level of radioactivity in the experiments.

DNA binding

Covalent linking of DNA can be obtained by fixation of its 5' end phosphate on the activated amino groups of the plastic (Zamatteo *et al*,1996) (Figure 1). Phosphorylated capture probes for Human Cytomegalovirus detection are denatured for 10 min at 100°C, cooled on ice (10 min), and diluted in ice-cold water (1.54 ng/µl). Ice cold 0.1 M 1-methylimidazole, pH 7.5, is added to obtain a final 1-methylimidazole concentration of 10 mM. The denatured DNA solution is dispensed into the microwells (75 µl/well, 0.7 pmol/well) standing on ice. A fresh solution of 0.04 M 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDC) in 10 mM 1-methylimidazole is added to each well (25 µl/well and they are incubated for 5 h at 50°C.
After incubation, the wells are washed three times with washing buffer (0.4 N NaOH, 0.25% Tween 20 at 50°C) 200 µl/well, then incubated 5 min with washing buffer, and finally washed three times again. Microwells are stored dried at 4°C. After the binding, the wells are cut and the amounts of ^{32}P-labelled DNA bound to the wells were measured by liquid scintillation counting.

2. Peptide Binding on primary amine support

Peptides can be chemically grafted on primary amino group via the free thiol group of a cysteine incorporated in the sequence using heterobinctional reagents such as succinimidyl 4-(N-maleimidomethyl) cycloexane-1-carboxilate (SMCC) (Yashida *et al*,1979: Hashida *et al*,1984) (figure 2) that can be purchased from Pierce Chemical Company (Rokford, USA). This crosslinker consists of an NHS ester and a maleimide group connected with a spacer arm. NHS esters react with primary amine, maleimides react with sulfhydryls.
Grafting can be evaluated by radiochemical assay if a tyrosine incorporated in the sequence is iodinated by oxidizing agent such as chloramine-T (Greenwood *et al*,1963).

Aminated microwells are incubated in a 6.5 10^{-2} mM solution of SMCC in 0.1M phosphate buffer (NHS esters react with primary amines at pH 7-9) during 1 h at room temperature. After 3 washes with phosphate buffer and 3 washes with water, the grafting is achieved by incubating cysteine -containing peptides (6.5 µM) overnight at room temperature, in 0.1 M phosphate buffer (maleimides react with SH groups at pH 6.5-7.5). After 3 washes in phosphate buffer, the wells are cut and the amount of coupled ^{125}I peptide is measured by a gamma irradiation counter.
Other bifunctional reagents such as N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) (Carlsson *et al*, 1978) can also be used.

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CARBOXYLATED

Surfaces with carboxylic groups covalently bound are dedicated to promote the covalent immobilization of compounds containing reactive free amino groups using the EDC mediated amination.

This kind of immobilization can overcome some of the limitations connected with physical adsorption of the molecules to the surfaces:

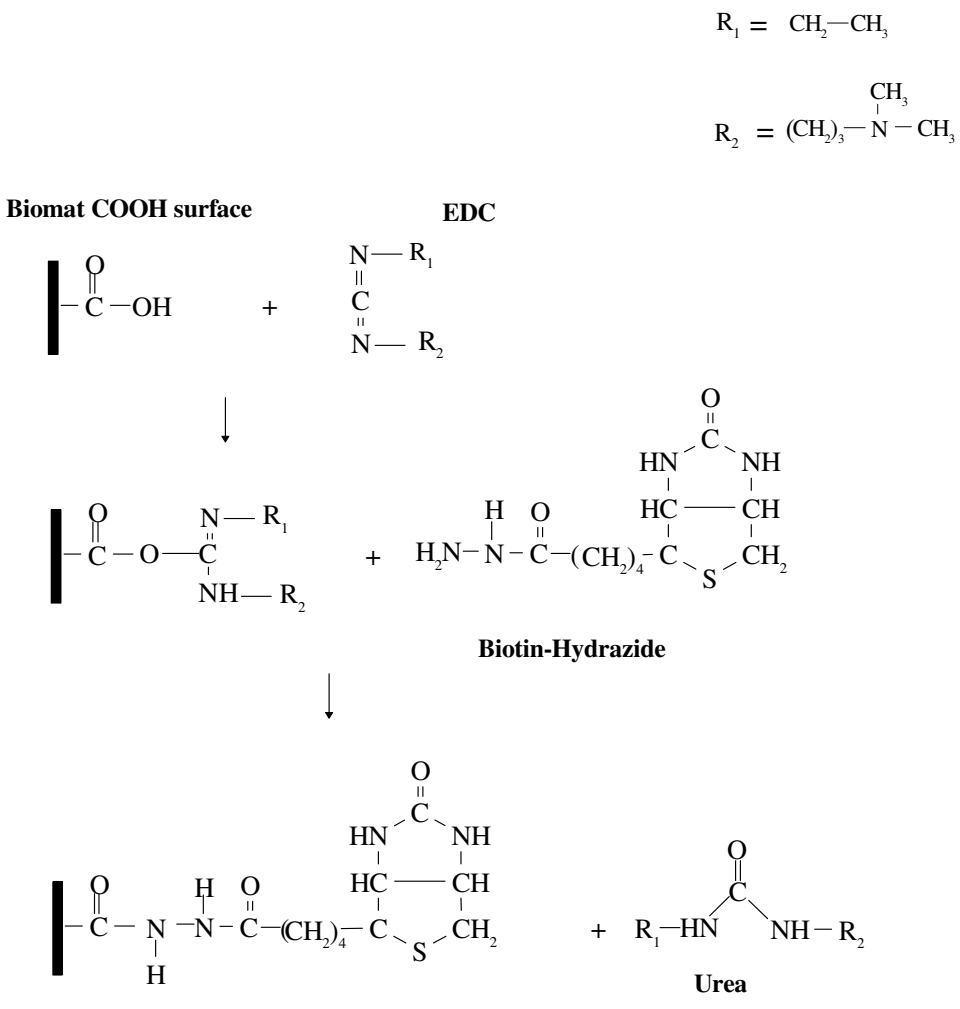
- immobilization of molecules which are bound weakly or not at all by physical adsorption, namely small peptides (M.W. 1000-5000 Da) drugs, toxins or hormones
- oriented immobilization of molecules in order to secure the integrity and accessibility of their specific sites
- increased storage stability compared with that of physical adsorption because of the reduced risk of spontaneous desorption

1. Coupling molecules, having an amino group, to Biomat COOH surface

The amino group presents in any molecules, such as peptides or proteins, binds to Biomat COOH through formation of amide bonds between the amino group presents in the molecule and the surface carboxylic group by the action of carbodiimide.

The figure 1 shows the reaction scheme for coupling of the hapten, biotin-hydrazide, through its available amino group.

Figure 1



Biotin-Hydrazide coupled to Biomat COOH surface

Preparation of reagents and buffers

Materials

Solid phase:	Biomat plates	MG04F-COOH MG01F-HB (high binding capacity)
Biotin-Hydrazide	Sigma	Cat. No. B 7639
1-Ethyl-3-(3 dimethylaminopropyl)-carbodiimide (EDC)	Sigma	Cat. No. E 1769
2-Morpholinoethanesulfonic acid (MES)	Fluka	Cat. No. 69889
Dimethylsulfoxide (DMSO)	Merck	Cat. No. 2931
Tween® 20	Merck	Cat. No. 822184
Streptavidin	BIO-SPA	Cat. No. S002-60
Streptavidin-peroxidase conjugate	BIO-SPA	Cat. No. SB01-61
BSA	Intergen	Cat. No. 3100
TMB peroxidase substrate	Kirkegard & Perry	Cat. No. 50-76-05

Biotin-hydrazide stock solution

Biotin-hydrazide	5 mg
DMSO	to 5 ml

Biotin-hydrazide solution 100 μg/ml

Biotin-hydrazide stock solution	1000 μl
EDC	10 mg
MES 0.1M pH 6.0	to 10 ml

Biotin-hydrazide solution 50 μg/ml

Biotin-hydrazide stock solution	500 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Biotin-hydrazide solution 10 μg/ml

Biotin-hydrazide stock solution	100 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Biotin-hydrazide solution 1.0 μg/ml

Biotin-hydrazide stock solution	10 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Biotin-hydrazide solution 0.5 μg/ml

Biotin-hydrazide stock solution	5 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Biotin-hydrazide solution 0.25 μg/ml

Biotin-hydrazide stock solution	2.5 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Biotin-hydrazide solution 0.1 μg/ml

Biotin-hydrazide stock solution	1.0 μl
EDC	10 mg
MES 0.1M pH 6.0	10 ml

Streptavidin-mix

Streptavidin	50 μg
Streptavidin-peroxidase	0.5 μg
PBS-BSA 1%	10 ml

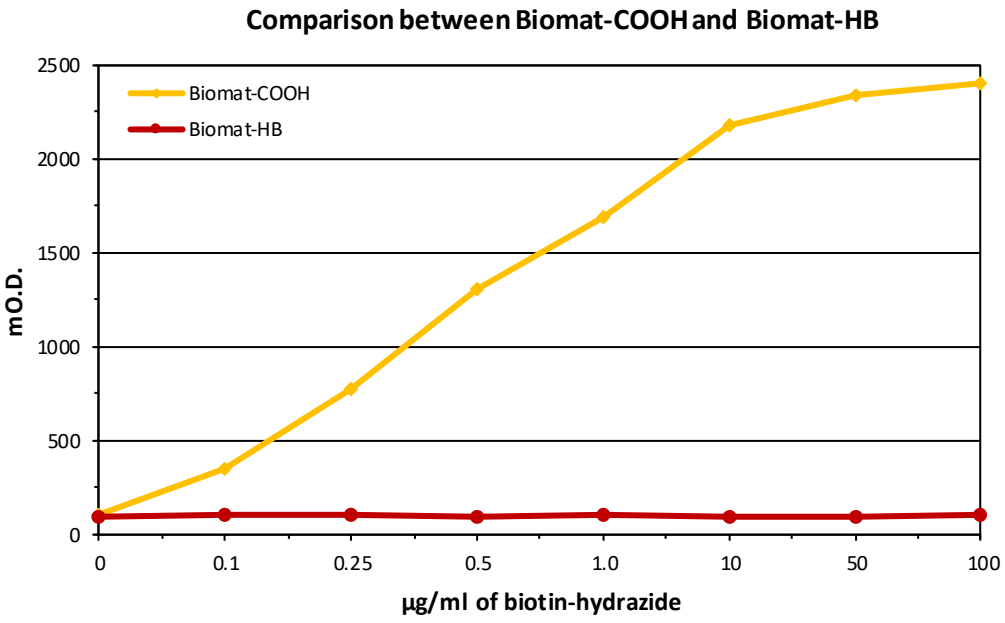
Experiment

1. Add 100 µl of biotin-hydrazide solutions 100-50-10-1-0.5-0.25-0.1 µg/ml and 100 µl 0.1 MES pH 6.0 as 0 µg/ml to the wells (carboxylated and HB not activated). Seal the wells with adhesive tape to prevent evaporation.
2. Incubate overnight at room temperature
3. Empty the wells and wash with 0.1 M PBS+0.05% Tween® 20 pH 7.2 four times
4. Add 100 µl of streptavidin- mix to each well and incubate 30 minutes at room temperature
5. Empty the wells and wash with 0.1 M PBS+0.05% Tween® 20 pH 7.2 four times
6. Add 100 µl of TMB substrate solution to each well and incubate 10 minutes at room temperature
7. Stop the substrate reaction by adding 100 µl of sulphuric acid 1 N and read the optical density values at 450 nm

Results

The results of this experiment (figure 2) clearly show that the molecule (biotin-hydrazide) is bound in a detectable way to the Biomat COOH whereas no detection could be obtained on Biomat HB.
The results indicate that a covalent coupling has taken place between the amino group in the biotin-hydrazide and the carboxylic group grafted on the Biomat COOH.
The results (data not displayed) show that without adding carbodiimide the covalent binding of biotin-hydrazide does not occur.

Figure 2



SECONDARY ANTIBODIES: GOAT ANTI MOUSE IgG Fcγ (Subclasses 1+2a+2b+3)

The Biomat product is a 96 well coated microplate with goat anti mouse IgG Fcγ (subclasses 1+2a+2b+3) and a protein to block non-specific binding sites and to maintain stable activity.
Affinity purified goat anti mouse IgG specifically binds the Fc region of mouse immunoglobulin subclasses 1,2a,2b and 3, with minimal cross-reaction to human, bovine and rabbit serum proteins.
These plates may be used as solid support for most sandwich ELISAs utilizing a mouse IgG capture and a non mouse IgG detection antibody. Other applications include competitive ELISA, IgG isotyping and hybridoma screening/selection.
These plates are ideal for binding assays when available antibodies are in low quantities or they denature and become inactive upon direct adsorption to polystyrene plates.

Features of goat anti mouse IgG antibody coated plates:

- prevent antibody denaturation as a result of direct adsorption to polystyrene
- unlike Protein A or G plates, these plates bind only to target IgG species
- these plates show a higher antibody-binding capacity than direct adsorption onto polystyrene when using diluted mouse IgG solutions

Product specifications

Coating

Affinity purified goat anti mouse IgG Fcγ (subclasses 1+2a+2b+3) 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 mouse IgG at a concentration of 1.0 µg/ml (100 ng/well) in an ELISA format using goat anti mouse IgG (H+L)-HRP as detector and TMB as substrate (see Figure 1 for data and experiment details).

The Biomat Goat anti mouse IgG Fcγ microplate shows a nominal **binding capacity of ~ 0.625 pmol/well of mouse IgG**

Sensitivity

Mouse IgG was detected at a concentration significantly above background in an ELISA format using goat anti mouse IgG (H+L)-HRP as detector and TMB as substrate (see Figure 1 for data and experiment details).

The Biomat Goat anti mouse IgG Fcγ microplate shows a **sensitivity of ~ 0.01 µg/ml of mouse IgG**.

Uniformity

Microplates show a **CV% less than 5** when used as a sandwich of mouse IgG in an ELISA format using goat anti mouse IgG (H+L)-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.

TECHNICAL NOTE N. 37

Binding capacity and sensitivity test

1. Add 100 µl of different concentrations of mouse IgG (from 0.025 to 4 µg/ml) to the wells of goat anti mouse IgG coated plate and incubate for 60 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 Goat anti-mouse IgG (H+ L)-HRP (Jackson ImmunoResearch code 115-035-003, diluted 1:150.000) 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 0.3 N and read the optical density values at 450 nm

The data show that a plateau has got starting with an IgG mouse concentration of 1.0 µg/ml.

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

- µg/well = 0.1 (100 ng/well)
- pmol/well= 0.625 (this result is calculated considering the IgG M.W. = 160.000 Da)

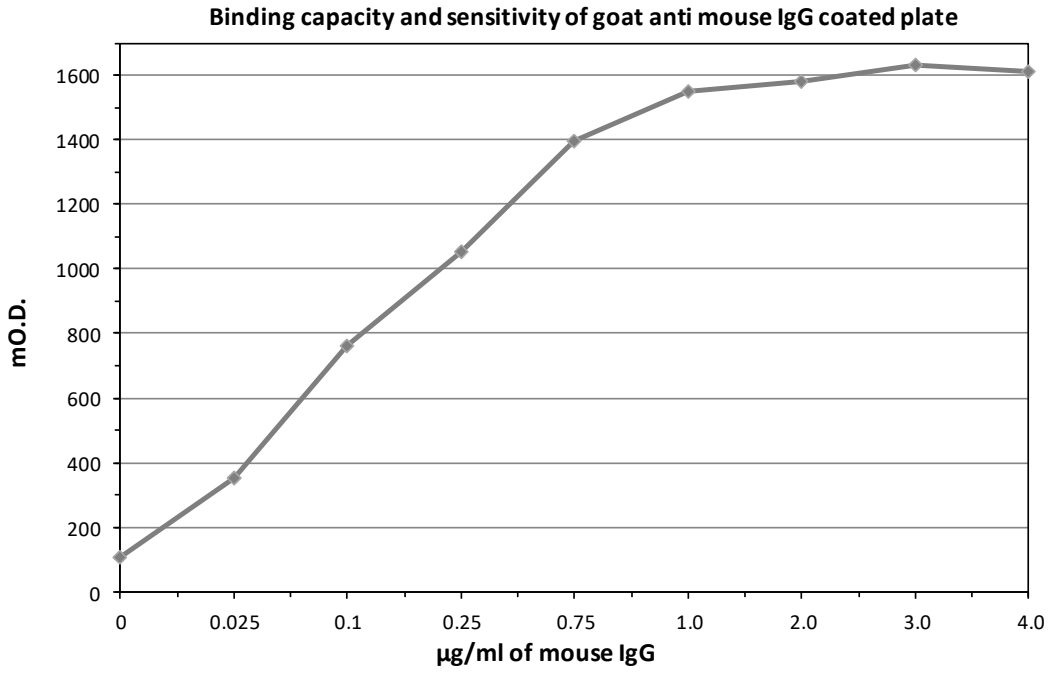
The microplate sensitivity was calculated as the lowest mouse IgG concentration higher than the mean optical

density plus 5 S.D. of 0 µg/ml mouse IgG concentration.

Our experiment gave the following results:

- 0 µg/ml mouse IgG optical density mean (coming from 8 replicates) = 0.108
- standard deviation = 0.014
- mean + 5 S.D. = 0.178
- sensitivity = 0.012 µg/well of mouse IgG

Figure 1



SECONDARY ANTIBODIES: GOAT ANTI RABBIT IgG Fc

The Biomat product is a 96 well coated microplate with goat anti rabbit IgG and a protein to block non-specific binding sites and to maintain stable activity.
Affinity purified goat anti rabbit IgG specifically binds the Fc region of rabbit immunoglobulins, with minimal cross-reaction to human serum proteins.
These plates are ideal for binding assays when available antibodies are in low quantities or they denature and become inactive upon direct adsorption to polystyrene plates.

Features of goat anti rabbit IgG antibody coated plates:

- prevent antibody denaturation as a result of direct adsorption to polystyrene
- unlike Protein A or G plates, these plates bind only to target IgG species
- these plates show a higher antibody-binding capacity than direct adsorption onto polystyrene when using diluted rabbit solutions

Product specifications

Coating

Affinity purified Goat anti rabbit IgG Fc 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 rabbit IgG at a concentration of 1.0 µg/ml (100 ng/well) in an ELISA format using goat anti rabbit IgG (H+L)-HRP as detector and TMB as substrate (see Figure 1 for data and experiment details).

The Biomat Goat anti rabbit IgG Fc microplate shows a nominal **binding capacity of ~ 0.625 pmol /well of rabbit IgG**

Sensitivity

Rabbit IgG was detected at a concentration significantly above background in an ELISA format using goat anti rabbit IgG (H+L)-HRP as detector and TMB as substrate (see Figure 1 for data and experiment details).

The Biomat Goat anti rabbit IgG Fc microplate shows a **sensitivity of ~ 0.01 µg/ml of rabbit IgG**.

Uniformity

Microplates show a **CV% less than 5** when used as a catcher of rabbit IgG in an ELISA format using goat anti rabbit IgG (H+L)-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.

TECHNICAL NOTE N. 38

Binding capacity and sensitivity test

7. Add 100 µl of different concentrations of rabbit IgG (from 0.025 to 4 µg/ml) to the wells of goat anti rabbit IgG coated plate and incubate for 60 minutes at room temperature
8. Empty the wells and wash with 0.1 M PBS pH 7.2, 0.05% Tween® 20 four times
9. Add 100 µl/well of Goat anti-rabbit IgG (H+L)-HRP (Jackson ImmunoResearch code 111-035-003, diluted 1:150.000) and incubate for 30 minutes at room temperature
10. Empty the wells and wash with 0.1 M PBS pH 7.2, 0.05% Tween® 20 four times
11. Add 100 µl/well of TMB substrate solution and incubate 15 minutes at room temperature
12. 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 rabbit concentration of 1.0 µg/ml.

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

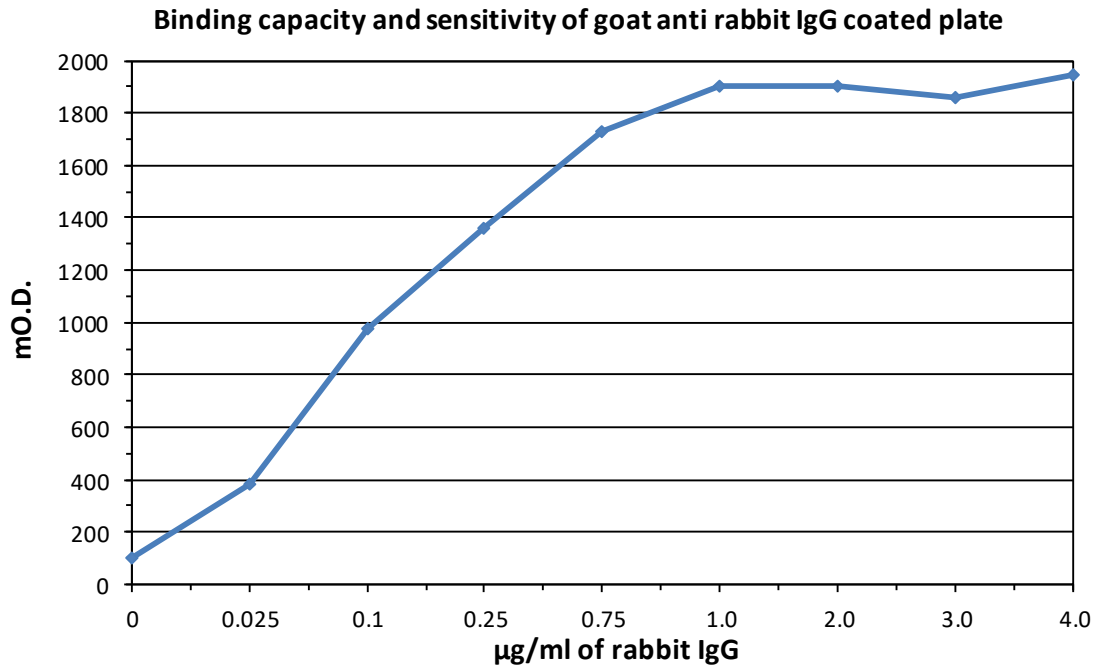
- µg/well = 0.1 (100 ng/well)
- pmol/well= 0.625 (this result is calculated considering the IgG M.W. = 160.000 Da)

The microplate sensitivity was calculated as the lowest rabbit IgG concentration higher than the mean optical density plus 5 S.D. of 0 µg/ml rabbit IgG concentration.

Our experiment gave the following results:

- 0 µg/ml rabbit IgG optical density mean (coming from 8 replicates) = 0.121
- standard deviation = 0.013
- mean + 5 S.D. = 0.186
- sensitivity = 0.010 µg/well of rabbit IgG

Figure 1

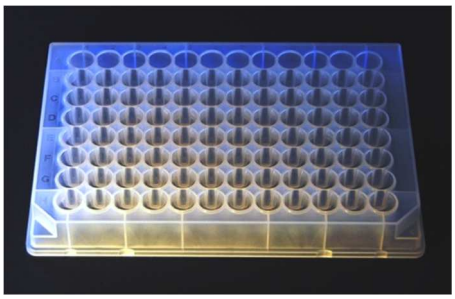


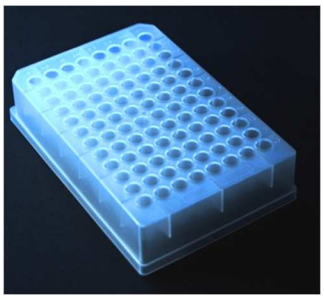
DEEPWELL PLATES

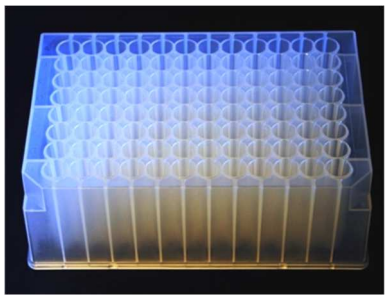
Biomat offers **Deep well plates** made of pure Polypropylene available in various volumes and shapes.

TECHNICAL FEATURES

- Volumes: 1.2 – 2.2 ml
- High-quality Polypropylene
- Good chemical and physical resistance: e.g. phenols, chloroform, DMSO and temperatures as low as -80ºC/-112ºF
- Compatible with most robotic samplers and automated liquid handling systems
- Alpha-numeric pattern identifies the wells, notched corners facilitate orientation
- Stackable for easy storage
- Cover mats are available in various materials







GLASS BOTTOM PLATES

Biomat glass bottom plates are suitable for:

- Fluorescence correlation spectroscopy (FCS)
- Confocal imaging
- Fluorescence polarization (FP)
- Cell-based assays
- Microarrays
- Laser-induced fluorescence (LIF)
- Cell culture

The technologies used in the manufacturing process:

- advanced robotics
- online gluing by advanced robotic
- clean room production

are addressed to obtain a product in which both the physical properties and the easy manipulation warrant the best performance for the user:

- superior flatness (planarity) - overall microplate flatness reduces scanning and imaging errors across the entire plane.
- no glue inclusion into the wells
- perfect edge glue deposition
- reduced auto fluorescence (less than 5%) which generates a higher signal-noise ratio

Formats

Glass bottom plates are available in different numbers of wells:

- 96
- 384
- 1536

Product features

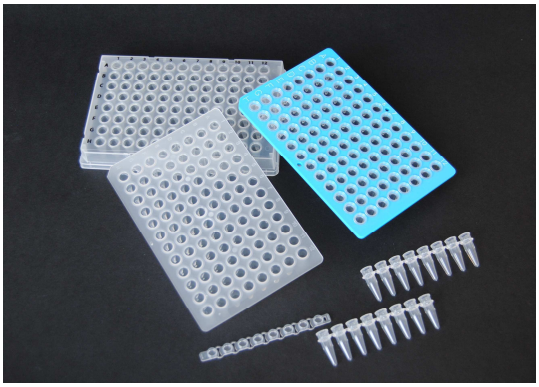
- Plates are designed and manufactured to comply with SBS standards
- Low auto fluorescence materials
- Increased working volume - Square well is ideal for high-throughput operations and delivers maximum volume
- Low base design - The low-base design ensures readability of all wells, including outer wells

Coated plates are available upon request: refer to surface features chapter.

COATED PCR PLATES AND 8 STRIP TUBES

Molecular Biology exploits several differentiated techniques that need different products, formats and polymers: from polystyrene plates to polypropylene PCR plates and tubes and from coated surfaces to tissue culture.

Biomat PCR plates are made in clear polypropylene coated with different molecules for your needs. Their thin walls *optimize the heat transfer from the block to the reaction solution*. while the rim of the wells is designed to *protect against cross-contamination*, allowing a reliable seal with the sealing mats tailored to the plates.



We offer three different types of plates suitable for Real-Time PCR:
– **96-well non-skirted PCR plates**, suitable for most commercially available thermal cyclers;
– **96-well semi-skirted PCR plates**, easily be labeled or tagged with a barcode:
– **96-well skirted PCR plates**: especially rigid, and optimal suited for use with automatic pipetting systems and for being transported.

PCR strip tubes are 8 connected 0.2 ml tubes made in clear polypropylene; they are thin walls that optimize the heat transfer from the block to the reaction solution.
Each PCR strip tubes has each numerically identified tube (from 1 to 8) for easy recognition.
The strips PCR are available with or without flat caps; they are easy to open and close without tools and are compatible with most popular thermal cycler blocks.

While polystyrene polymer is easily subject to binding capacity improvement and is able to bind a wide range of molecules, the polypropylene is an inert polymer.

Thanks to a proprietary method for improving the binding capacity of polypropylene, now PCR 8 tubes strips with coated molecules are offered:

- Streptavidin
- HB streptavidin
- Protein A
- Protein G
- Protein A/G
- Maleimide
- Carboxylated

or with different molecules upon request.

STREPTAVIDIN COATED PCR PRODUCTS

Streptavidin coated surfaces offer a powerful and universal instrument for binding any biotinylated molecule (Proteins-Peptides-Polysaccharides-Oligonucleotides-DNA fragments-etc.)
Streptavidin is a tetrameric protein (M.W. 60 kDa) with very high affinity for biotin (Ka=10^{-15} M); the bond is the strongest known non-covalent biological interaction.
Biotin is a small molecule which can be conjugated to many proteins without losing or altering their activity, each protein can bind many biotin molecules.
Since each subunit of streptavidin binds one molecule of biotin, the resulting effect is a great increase of the sensitivity of the assay.
The streptavidin-biotin bonding main features

- stability
- specificity
- affinity

make it useful for special applications of molecules which do not offer reliable bonding by passive adsorption or adsorb in a unfavorable orientation.

Product specifications

Coating

Streptavidin is coated using 100 µl/tube. The final product is post-coated (blocked) for low non specific binding and long-term stability.

Uniformity

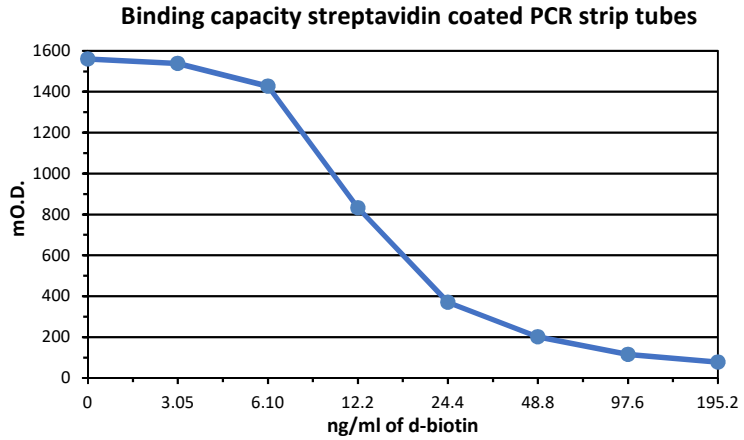
Streptavidin PCR plates and PCR 8 strip tubes show a **CV% less than 5** when used as a catcher of biotin-HRP as detector in an ELISA format using TMB as substrate.

Shelf life

2 years from date of manufacture.

Binding capacity streptavidin coated PCR 8 strip tubes

Streptavidin coated PCR 8 strip tubes were incubated with biotin solutions (from 0 to 195.2 ng/ml) containing 1.3 ng/ml of biotinylated peroxidase for 30' R.T. After washing step, the streptavidin PCR 8 strip tubes were incubated with TMB and blocked with sulphuric acid 1N. The O.D. values were read at 450 nm.



The Biomat Streptavidin PCR 8 strip tubes show a nominal **binding capacity of ~ 5 - 6 pmol d-biotin/tube.**

HIGH BINDING STREPTAVIDIN COATED PCR PRODUCTS

Streptavidin HB PCR plates and PCR 8 strip tubes are a powerful and universal instrument for binding any biotinylated molecule (Proteins-Peptides-Polysaccharides-Oligonucleotides-DNA fragments-etc.).
Biotin is a small molecule which can be conjugated to many proteins without losing or altering their activity, each protein can bind many biotin molecules.
Since each subunit of streptavidin binds one molecule of biotin, the resulting effect is a great increase of the sensitivity of the assay.
Unlike the normal Streptavidin plates or Streptavidin PCR 8 strip tubes, these PCR plates or PCR strip tubes are particularly useful in competitive tests to measure biotinylated low molecular weight molecules.

Product specifications

Coating

Streptavidin HB is coated using 100 µl/tube. The final product is post-coated (blocked) for low non specific binding and long-term stability.

Uniformity

Streptavidin HB PCR plates and PCR 8 strip tubes show a **CV% less than 5** when used as a catcher of biotin-HRP as detector in an ELISA format using TMB as substrate.

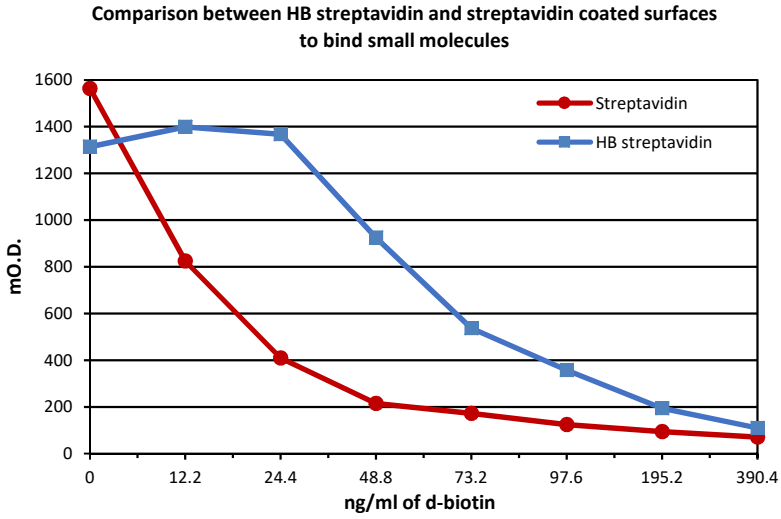
Shelf life

2 years from date of manufacture.

TECHNICAL NOTE N. 33

1. Comparison between HB streptavidin and streptavidin coated surfaces to bind small molecules (biotin, MW= 244 Da)

Streptavidin HB and streptavidin coated PCR 8 strip tubes were incubated with biotin solutions (from 0 to 390.4 ng/ml) containing 1.3 ng/ml of biotinylated peroxidase for 30' R.T.
After a washing step, the wells were incubated with TMB and blocked with sulphuric acid 1N. The O.D. values were read at 450 nm.



The Biomat HB Streptavidin PCR 8 strip tubes show a nominal **binding capacity of > 20 pmol d-biotin/tube.**

PROTEIN A – G – A/G COATED PCR PRODUCTS

PROTEIN A COATED PCR PRODUCTS

The Biomat products are a PCR plates and a PCR 8 strip tubes coated with recombinant Protein A and a protein to block non-specific binding sites and to maintain stable activity.
Protein A coated surface is designed for capture specific and sterically oriented IgG applied directly or as antigen/antibody complex. Among its applications there are: separation of IgG from other immunoglobulins or contaminants, separation of antigen-antibodies complex and isolation and analysis of fusion proteins.
Protein A specifically binds the Fc region of immunoglobulins of many mammalian species (see table 1 below), with an orientation that allows the $F(ab)_2$ binding sites to be freely available for efficient binding to epitope. When coated onto PCR 8 strip tubes, the Protein A can securely capture IgG applied directly or as antigen/antibody complexes.
Protein A PCR products 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

Product specifications

Coating

Recombinant Protein A (M.W. 38.9 kDa), from *Staphylococcus aureus subsp. Aureus*, expressed in *E. coli*, is coated using 100 µl/tube. The products is post-coated (blocked) for low non specific binding and long-term stability.

Uniformity

Protein A PCR plates and PCR 8 strip tubes 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.

Shelf life
1 year from date of manufacture.

PROTEIN G COATED PCR PRODUCTS

The Biomat products are a PCR plates and a PCR 8 strip tubes coated 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 (see table 1 below), with an orientation that allows the $F(ab)_2$ binding sites to be freely available for efficient binding to epitope. When coated onto PCR 8 strip tubes, 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

Coating
Recombinant Protein G (M.W. 26.1 kDa), from *Streptococcus sp.*, expressed in *E. coli*, is coated using 100 µl/tube. The PCR 8 strip tubes are post-coated (blocked) for low non specific binding and long-term stability.

Uniformity

Protein G PCR plates and PCR 8 strip tubes 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.

Shelf life

1 year from date of manufacture.

PROTEIN A/G COATED PCR PRODUCTS

The Biomat products are a PCR plates and a PCR 8 strip tubes coated 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 versatile tool. The binding dependency to pH of Protein A/G is lower than Protein A but has the additive properties of Protein A and G together.
The Protein A/G binds to human IgG, IgA, IgM; it binds to all subclasses of mouse IgG excluding mouse IgA, IgM and serum albumin (see table 1 below). When coated onto PCR 8 strip tubes, the Protein A/G can securely to be used in purification and detection of mouse monoclonal IgG antibodies with no interference from IgA, IgM and serum albumin.

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

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 100 µl/tube. The PCR products are post-coated (blocked) for low non specific binding and long-term stability.

Uniformity

Protein A/G PCR plates and PCR 8 strip tubes show a **CV% less than 5**.

Shelf life

1 year from date of manufacture.

Table 1. Binding affinities of recombinant Protein A, G and A/G for antibodies class.
(The table 1 gives an overview of binding strengths of protein A, G and A/G to different species and subclasses)

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

S: strong binding; M: medium binding; W: weak binding; N: no binding

MALEIMIDE COATED PCR PRODUCTS

The Biomat products are a PCR plates and a PCR 8 strip tubes coated with maleimide and treated to block non-specific binding sites and to maintain stable activity.
Maleimide coated surfaces offer a powerful instrument for binding biomolecules containing free sulfhydryl groups (e.g. peptides that contain a terminal cysteine or thiol containg haptens), or reducible disulfide bonds that are difficult to coat onto polystyrene plates. These coated products are a very useful tool for assays requiring site-directed orientation of particular biomolecules.
At pH 6.5-7.5 maleimide reacts with free sulfhydryl groups to yeld stable bonds, while the reaction with amine becomes significant at pH > 7.5.
If sulfhydryl-containing peptides and proteins oxidize in solution and form disulfide bonds, they must be preventively reduced to free sulfhydryls for allowing interaction with maleimide.

Product specifications

Coating

A derived maleimide is coated using 100 µl/tube. The PCR products are post-coated (blocked) for low non specific binding and long-term stability.

Storage and Stability

3 months from date of manufacture.

CARBOXYLATED SURFACE PCR PRODUCTS

Surfaces with carboxylic groups covalently bound are dedicated to promote the covalent immobilization of compounds containing reactive free amino groups using the EDC mediated amination.
This kind of immobilization can overcome some of the limitations connected with physical adsorption of the molecules to the surfaces:

- immobilization of molecules which are bound weakly or not at all by physical adsorption, namely small peptides (M.W. 1000-5000 Da) drugs, toxins or hormones
- oriented immobilization of molecules in order to secure the integrity and accessibility of their specific sites

Product specifications

Treatment

PCR products are treated with special techniques so that they are attached to the surface of carboxyl groups.

Storage and Stability

2 years from date of manufacture.

COATED 12 x 75 mm TUBES

Biomat 12×75 mm coated tubes offer a reliable response to the needs of kit manufacturers and researchers.

12 x 75 mm tubes are made in high quality clear polystyrene.
They are 75 mm long and have an outside diameter of 12 mm for a total volume of 5 mL. These tubes have a bottom round.
They are ideal for solid phase immunotechniques such as IRMA, ELISA and ILMA.

STREPTAVIDIN COATED TUBES

Streptavidin coated surfaces offer a powerful and universal instrument for binding any biotinylated molecule (Proteins-Peptides-Polysaccharides-Oligonucleotides-DNA fragments-etc.)
Streptavidin is a tetrameric protein (M.W. 60 kDa) with very high affinity for biotin (Ka=10^{-15} M); the bond is the strongest known non-covalent biological interaction.
Biotin is a small molecule which can be conjugated to many proteins without losing or altering their activity, each protein can bind many biotin molecules.
Since each subunit of streptavidin binds one molecule of biotin, the resulting effect is a great increase of the sensitivity of the assay.
The streptavidin-biotin bonding main features

- stability
- specificity
- affinity

make it useful for special applications of molecules which do not offer reliable bonding by passive adsorption or adsorb in a unfavorable orientation.

Product specifications

Coating

Streptavidin is coated using 500 µl/tube. The final product is post-coated (blocked) for low non specific binding and long-term stability.

Shelf life
3 years from date of manufacture.

HIGH BINDING STREPTAVIDIN COATED TUBES

Streptavidin HB 12x75 mm tubes are a powerful and universal instrument for binding any biotinylated molecule (Proteins-Peptides-Polysaccharides-Oligonucleotides-DNA fragments-etc.).
Unlike the normal Streptavidin tubes, this product is particularly useful in competitive tests to measure biotinylated low molecular weight molecules.

Product specifications

Coating

Streptavidin HB is coated using 500 µl/tube. The final product is post-coated (blocked) for low non specific binding and long-term stability.

Shelf life
2 years from date of manufacture.

TISSUE CULTURE TREATED ARTICLES

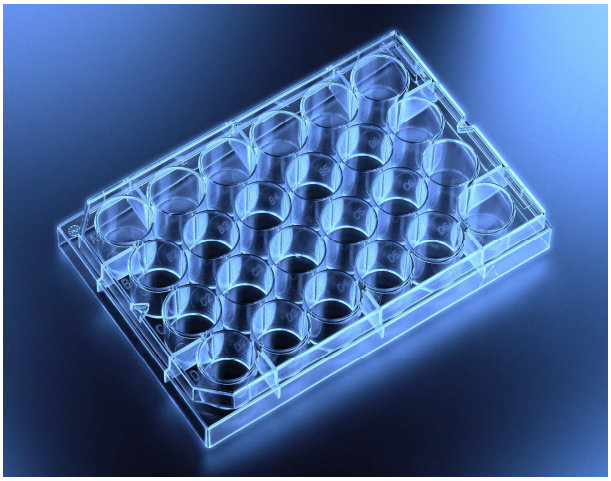
Biomat TC surface treatment is based on physical surface modifications.
While it was developed and mainly used for PS articles, we obtained good results also TC treating other polymers as COP and COC.

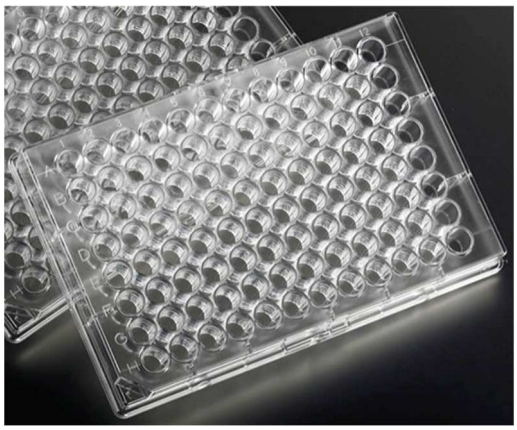
- Available on 24-96 well plates
- Customers' own articles

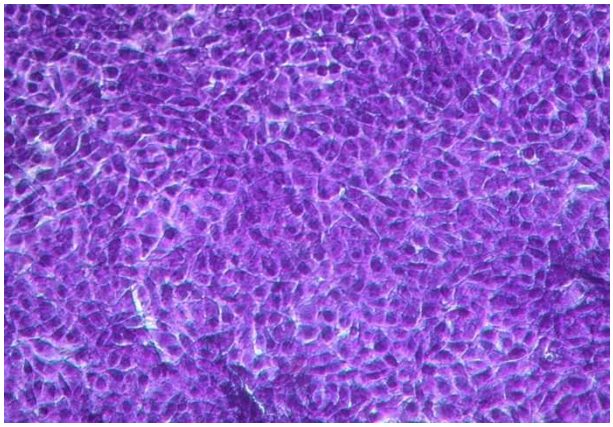
PLASMA

General features

- The surface which offers optimal support for cell growth is obtained by proprietary vacuum plasma treatment
- **Vacuum plasma treatment**, thanks to its strictly controlled parameters, warrants consistency of behavior of the surface
- The surface chemistry offers a uniform surface with both hydrophilic and negative charge properties





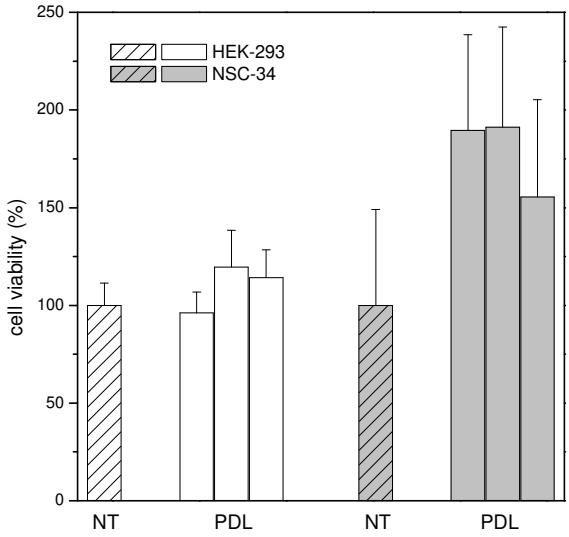


Cell growth on microplate's surface (L929 blue stained)

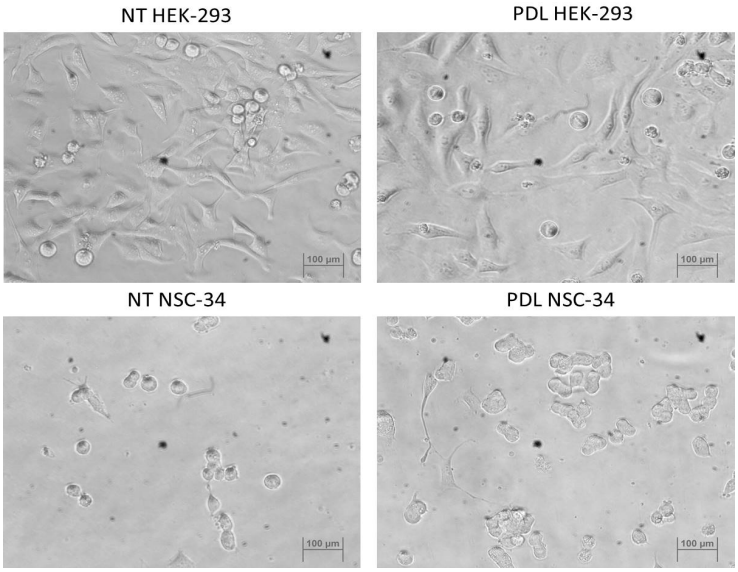
POLY- D-LYSINE COATED SURFACE

Poly-D-Lysine (PDL) Coated Plates: these microplates are coated with a synthetic PDL molecular weight range of 70-150 kDa. By coating the negatively charged polystyrene surface with PDL, the resulting positively charged surface has been shown to enhance cell attachment. Many cell types adhere better to this surface and are less dependent on the presence of serum proteins.

Biomat PDL microplates were tested with very positive results for: (i) cell proliferation and viability (ii) cell morphology and differentiation (iii) maintenance of sterility over aging. Standard optical microscopy techniques and cell proliferation assay (MTT) were employed.



Biomat PDL treated microplates exhibit high lot-to-lot consistency and improved performance for cell proliferation and viability with respect to the non-treated (NT) microplates. The well-to-well consistency was proven on three different microplates belonging to three different lots, showing high reproducibility in cell viability. Two cell lines were cultured on PDL and NT microplates with improved growing performances on PDL microplates in particular for the NSC-34 cell line (Neuroblastoma Spinal Cord). Cell viability was measured with MTT assay.
Aging of PDL microplates was also monitored, showing that PDL microplates maintain good performances up to two years in standard growing conditions.



Adhesion of both cell lines (HEK-293 and NSC-34) cultured on PDL microplates was improved and cell morphology maintained. Images were taken with an optical microscope with 20X objective and in bright field. Scale bar is 100 µm.

ACCESSORIES

In order to help our customer to rely on a single supplier of high quality products, we offer a line of accessories and components for manufacturers and researchers.

BARRIER BAGS
Biomat offers re-sealable barriers bags with zip
Opaque Barrier Film Inner Layer complies with food and pharmaceutical regulations (FDA 21 CFR177 1520).
Construction/Composition:

12 microns	PET (Polyester) Bonding Layer
8 microns	Aluminum Foil Bonding Layer
100 microns	White/Clear LLDPE Film Layer

STRIP REMOVER
A simple device to help the user in removing the breakable strips from their frame

SILICA GEL
5 grams bags

SERVICES

Thanks to more than 25 years in the industry Biomat also offers its customers the following services:

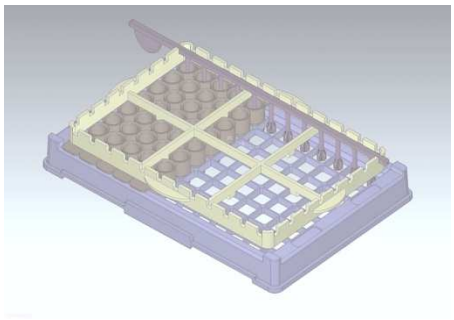
- COATINGS
- SURFACE TREATMENTS: from COATINGS to ACTIVATIONS
- ENGINEERING: PRODUCT DEVELOPMENT SERVICE

Biomat offers a fast and reliable service to the customers, cooperating with companies and institutions worldwide:

- Making surface modifications
- Offering **coating service**: thanks to our experience, we are able to work both on standard plates and on customer's own articles. The service starts from the process setup to the supply of the final product, tested, packed and supplied with CQ certificate
- **Developing** new products jointly with the customer
- Offering an advanced technical support for projects, analysis and engineering

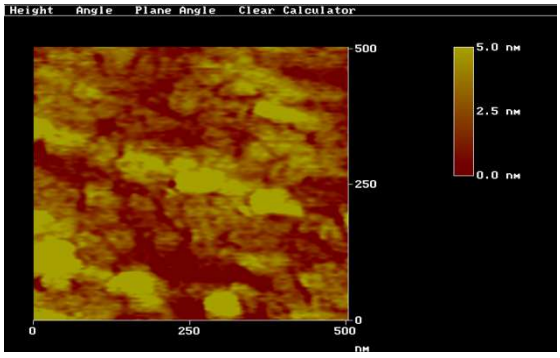
Our expertise covers a wide range of techniques for the modifications of polymer surfaces:

- Plasma (glow discharge)
- Chemical modification
- Biological coating



Project development

Advanced laboratory equipment



Surface analysis

Plasma reactor

SURFACE TREATMENTS ON CUSTOMERS' ITEMS

Biomat's mission is to support life science manufacturers and researchers supplying them with the proper surface for their application.

The plasma treatments are particularly suitable to be employed in the biomedical field thanks to their characteristics
Every type of surface treatment is studied and developed for each item or customer in order to obtain the surface properties that are needed for each specific application.

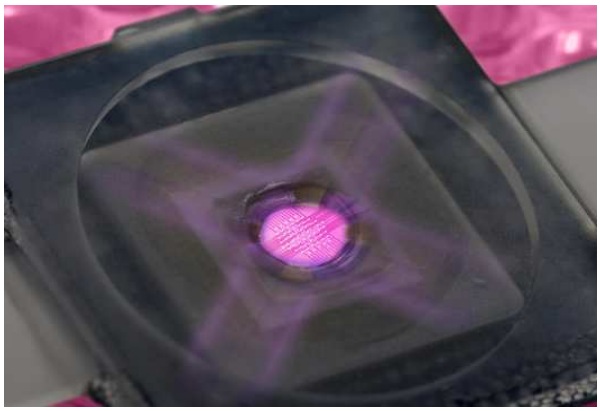
Plasma treatments are suitable for:

- preparing hydrophilic or hydrophobic surfaces suitable for biomedical and industrial applications
- improving the adhesion of inks, paints and glues
- surface cleaning / etching

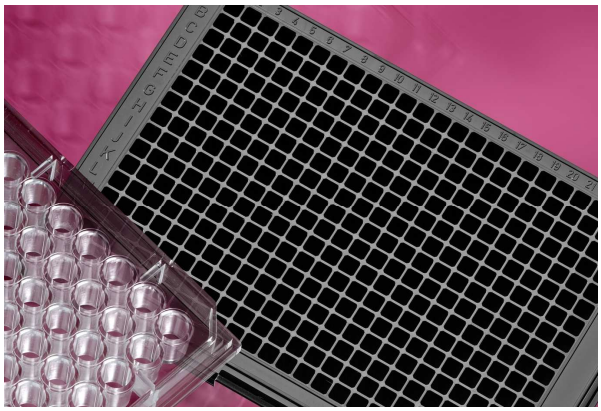
A wide range of articles have shown an improvement of their performance related to the biological activity thanks to the plasma treatment.

We are well aware that, with the evolution of the assay technology, many users are developing their own devices. Therefore, besides offering our own microplates, we offer the application of the same range of surfaces on the customers' own articles, no matter which is their shape or final destination.

Surface treatment service



Technology transfer



Examples of application are:

- **Biochip**
- **Filters**
- **Cuvettes**
- **Special devices**

which are surface modified according with the needs of the customer.
The performance that the user expects, the shape of the part, the caracteristics of the material together with all the processing and storage features, are taken into consideration and the treatment is developed step by step in cooperation with the user in order to optimize it.

THE PLASMA

Plasma equipment and process can be described in few words:

the equipment consists in a vacuum chamber with one or more gas inlet (equipped with proper dosing systems), two electrodes connected to a RF (or different frequency) generator are located within the chamber in order to supply to the gas that flows between them the energy that excites its molecules generating the plasma.
The process runs this way:

1. articles to be treated are placed within the chamber
2. a fixed degree of vacuum is reached
3. a fixed amount of the chosen gas(es) is made to flow through the chamber and excited by the electrical discharge creating the PLASMA
4. the reactive species of plasma react with the surface of the device modifying its properties

The process works in short times (from seconds to minutes) practically at room temperature affecting only the surface of the parts that are treated.
One of the most common applications of the **COLD PLASMA** technology is the modification of the wettability of the polymers.
Polymers usually show a not wettable surface (contact angle > 90°).

This property means that, whenever we need to have a good adhesion of anything to the surface, we encounter a poor response and we need to treat the surface in order to have an acceptable behavior.
Examples of applications where a good wettability is important are, for example:

- adhesion of biological molecules
- glueing
- inking
- painting

Since every type of polymer shows different properties, the treatment must be studied and applied for the specific material, previously taking into consideration such features as:

- the raw material
- the shape of the part
- the application

applying to the process the specific parameters:

- **TYPE OF GAS(ES)**
- **TIME**
- **POWER**

and, after the treatment, testing the treated parts for:

- efficiency of the treatment
- uniformity
- durability of its effects during storage

cooperating with the user in order to find the best treatment both for technical and economical features.

Biomat studies, develops and applies surface treatments with its plasma reactor installed in its facility.

PRODUCT CATALOGUE

Description	*Code*
96 WELLS PLATE 12 X 8 FRAME	MT
96 WELLS PLATE SINGLE WELL HOLDING FRAME	MG
96 WELL SOLID PLATE	MC
TUBES	TS
MATERIAL	TRANSPARENT POLYSTYRENE Unless otherwise indicated Strips and plates in black or white polystyrene are available upon request

TISSUE CULTURE PRODUCTS

Code	*Description*	*Quantity per pack*	*Quantity per case*
MC23FL-24-TC/S	24 well plate Tissue Culture treated- sterile with lid	1	80
MC23F-24-TC	24 well plate Tissue Culture treated	20	160
MC23FL-96-TC/S	96 well plate Tissue Culture treated- sterile with lid	1	160
MC23F-96-TC	96 well plate Tissue Culture treated	25	200
MC23FL-384-TC/S	384 well plate Tissue Culture treated- sterile with lid	1	160
MC23F-384-TC	384 well plate Tissue Culture treated	25	200
OTHER FORMATS AVAILABLE UPON REQUEST			

POLY-D-LYSINE COATED (MW 70-150 kDa) + S (sterile)			
Code	*Description*	*q.ty/pack*	*q.ty/case*
MC24F-LYS-D/S	96 wells plate + lid	1	50 or 100

TRANSPARENT BOTTOM PLATES

Code	*Description*	*q.ty/pack*	*q.ty/case*
MCB-GB–96	96 wells plate Black PS - Glass Bottom	5	40
MCB-GB-384	384 wells plate Black PS - Glass Bottom	5	40
MCB-GB-1536	1536 wells plate Black PS - Glass Bottom	5	40

HTS PLATES

Code	*Description*	*q.ty/pack*	*q.ty/case*
MC0F-96	96 wells plate Clear PS	25	200
MC0F-384	384 wells plate Clear PS	25	200
MCW0F-96	96 wells plate White PS	25	200
MCW0F-384	384 wells plate White PS	25	200
MCB0F-96	96 wells plate Black PS	25	200
MCB0F-384	384 wells plate Black PS	25	200

POLYPROPYLENE PLATES - STORAGE

Code	*Description*	*q.ty/pack*	*q.ty/case*
MC0V-PP450	96 wells plate PP V bottom 450 µl	25	200
MC0U-PP1200	1.2 ml Deep Well Plate PP 96 Round Wells U-Bottom low profile-stackable	48	192
MC0U-PP2200	2,2 ml Deep-well plates PP 96 Round Wells U-Bottom	24	96
OTHER FORMATS AVAILABLE UPON REQUEST			

MATS FOR DEEPWELL PLATES MATERIAL: TPA			
Code	***Description***		
MA-1200-TPA	Mat Caps for 1,2 ml Round Well Plates		

IMMUNOASSAY PRODUCTS

MEDIUM BINDING CAPACITY					
Clear	***White***	***Black***	***Description***	***q.ty/pack***	***q.ty/case***
MT0F2-MB	MTW0F2-MB	MTB0F2-MB	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame	25	200
MG0F-MB	MGW0F-MB	MGB0F2-MB	96 wells plate - 1x8 flat bottom wells strips on single well holding frame	25	200
MC0F-MB	MCW0F-MB	MCB0F-MB	96 wells plate - 96 wells plate - flat bottom well capacity 400 µl	25	200

HIGH BINDING CAPACITY HB8					
Clear	***White***	***Black***	***Description***	***q.ty/pack***	***q.ty/case***
MT01F2-HB8	MTW01F2-HB8	MTB01F2-HB8	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame	25	200
MG01F-HB8	MGW01F-HB8	MGB01F2-HB8	96 wells plate - 1x8 flat bottom wells strips on single well holding frame	25	200
MC01F-HB8	MCW01F-HB8	MCB01F-HB8	96 wells plate - flat bottom well capacity 400 µl	25	200
STANDARD WELL CAPACITY 350 ul, PLATES WITH 400 ul WELL CAPACITY ARE AVAILABLE UPON REQUEST					

SPECIAL SURFACES PRODUCTS

No Binding capacity			
Clear	***White***	***Black***	***Description***
MT03F2-NB	MTW03F2-NB	MTB03F2-NB	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG03F-NB	MGW03F-NB	MGB03F-NB	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC03F-NB	MCW03F-NB	MCB03F-NB	96 wells plate – flat bottom

Plates: q.ty/pack = 25 q.ty/case = 100 or 200

COATED PLATES **(packed 1 plate into single barrier bag resealable)**

q.ty/pack = 1 q.ty/case = 50 or 100

Biotin coated			
Clear	***White***	***Black***	***Description***
MT15F2-BIO	MTW15F2-BIO	MTB15F2-BIO	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG15F-BIO	MGW15F-BIO	MGB15F2-BIO	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC15F-BIO	MC1W5F-BIO	MCB15F-BIO	96 wells plate - flat bottom

Calmodulin coated			
Clear	***White***	***Black***	***Description***
MT14F2-CAL	MTW14F2-CAL	MTB14F2-CAL	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG14F-CAL	MGW14F-CAL	MGB14F2-CAL	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC14F-CAL	MCW14F-CAL	MCB14F-CAL	96 wells plate - flat bottom

Concanavalin A coated			
Clear	***White***	***Black***	***Description***
MT07F2-CON A	MTW07F2-CON A	MTB07F2-CON A	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG07F-CON A	MGW07F-CON A	MGB07F2-CON A	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC07F-CON A	MCW07F-CON A	MC07F-CON A	96 wells plate - flat bottom

Heparin catcher 0.01 to 2.0 U/ml			
Clear	***White***	***Black***	***Description***
MT18F2-HC1	MTW18F2-HC1	MTB18F2-HC1	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG18F-HC1	MGW18F-HC1	MGB18F2-HC1	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC18F-HC1	MCW18F-HC1	MCB18F-HC1	96 wells plate - flat bottom

Heparin catcher 0.5 to 40.0 U/ml			
Clear	***White***	***Black***	***Description***
MT19F2-HC2	MTW19F2-HC2	MTB19F2-HC2	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG19F-HC2	MGW19F-HC2	MGB19F2-HC2	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC19F-HC2	MCW19F-HC2	MCB19F-HC2	96 wells plate - flat bottom

Heparin catcher 2.0 to 160.0 U/ml			
Clear	***White***	***Black***	***Description***
MT20F2-HC3	MTW20F2-HC3	MTB20F2-HC3	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG20F-HC3	MGW20F-HC3	MGB20F2-HC3	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC20F-HC3	MCW20F-HC3	MCB20F-HC3	96 wells plate - flat bottom

Jacalin coated			
Clear	***White***	***Black***	***Description***
MT10F2-JAC	MTW10F2-JAC	MTB10F2-JAC	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG10F-JAC	MGW10F-JAC	MGB10F2-JAC	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC10F-JAC	MCW10F-JAC	MCB10F-JAC	96 wells plate – flat bottom

Maleimide coated			
Clear	***White***	***Black***	***Description***
MT22F2-MAL	MTW22F2-MAL	MTB22F2-MAL	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG22F-MAL	MGW22F-MAL	MGB22F2-MAL	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC22F-MAL	MCW22F-MAL	MCB22F-MAL	96 wells plate – flat bottom

Neutravidin coated			
Clear	***White***	***Black***	***Description***
MT21F2-NA	MTW21F2-NA	MTB21F2-NA	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG21F-NA	MGW21F-NA	MGB21F2-NA	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC21F-NA	MCW21F-NA	MCB21F-NA	96 wells plate - flat bottom

Poly Arginine coated			
Clear	***White***	***Black***	***Description***
MT13F2-AR	MTW13F2-AR	MTB13F2-AR	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG13F-AR	MGW13F-AR	MGB13F2-AR	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC13F-AR	MCW13F-AR	MCB13F-AR	96 wells plate - flat bottom

Poly-L-Lysine coated (M.W. 70 - 150 kDa)			
Clear	***White***	***Black***	***Description***
MT12F2-LYS-L	MTW12F2-LYS-L	MTB12F2-LYS-L	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG12F-LYS-L	MGW12F-LYS-L	MGB12F2-LYS-L	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC12F-LYS-L	MCW12F-LYS-L	MCB12F-LYS-L	96 wells plate - flat bottom

Protein A coated			
Clear	***White***	***Black***	***Description***
MT06F2-PA	MTW06F2-PA	MTB06F2-PA	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG06F-PA	MGW06F-PA	MGB06F2-PA	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC06F-PA	MCW06F-PA	MCB06F-PA	96 wells plate – flat bottom

Protein A/G coated			
Clear	***White***	***Black***	***Description***
MT08F2-PAG	MTW08F2-PAG	MTB08F2-PAG	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG08F-PAG	MGW08F-PAG	MGB08F2-PAG	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC08F-PAG	MCW08F-PAG	MCB08F-PAG	96 wells plate - flat bottom

Protein G coated			
Clear	***White***	***Black***	***Description***
MT09F2-PG	MTW09F2-PG	MTB09F2-PG	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG09F-PG	MGW09F-PG	MGB09F2-PG	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC09F-PG	MCW09F-PG	MCB09F-PG	96 wells plate - flat bottom

Streptavidin coated			
Clear	***White***	***Black***	***Description***
MT0STF2-SA5/200	MTW0STF2-SA5/200	MTB0STF2-SA5/200	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG0STF-SA5/200	MGW0STF-SA5/200	MGB0STF2-SA5/200	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC0STF-SA5/200	MCW0STF-SA5/200	MCB0STF-SA5/200	96 wells plate - flat bottom

High Binding Streptavidin coated			
Clear	***White***	***Black***	***Description***
MTSTDF2-SB75/100	MTWSTDF2-SB75/100	MTBSTDF2-SB75/100	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MGSTDF-SB75/100	MGWSTDF-SB75/100	MGBSTDF2-SB75/100	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MCSTDF-SB75/100	MCWSTDF-SB75/100	MCBSTDF-SB75/100	96 wells plate - flat bottom

Wheat Germ coated			
Clear	***White***	***Black***	***Description***
MT11F2-WG	MTW11F2-WG	MTB11F2-WG	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG11F-WG	MGW11F-WG	MGB11F2-WG	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC11F-WG	MCW11F-WG	MCB11F-WG	96 wells plate - flat bottom

Secondary antibodies coated – Anti Mouse IgG			
Clear	***White***	***Black***	***Description***
MT25F2-aM	MTW25F2-aM	MTB25F2-aM	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG25F-aM	MGW25F-aM	MGB25F2-aM	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC25F-aM	MCW25F-aM	MCB25F-aM	96 wells plate - flat bottom

Secondary antibodies coated – Anti Rabbit IgG			
Clear	***White***	***Black***	***Description***
MT26F2-aR	MTW26F2-aR	MTB26F2-aR	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG26F-aR	MGW26F-aR	MGB26F2-aR	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC26F-aR	MCW26F-aR	MCB26F-aR	96 wells plate - flat bottom

ACTIVATED PLATES

Primary amino groups			
Clear	***White***	***Black***	***Description***
MT02F2-AM1	MTW02F2-AM1	MTB02F2-AM1	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG02F-AM1	MGW02F-AM1	MGB02F-AM1	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC02F-AM1	MCW02F-AM1	MCB02F-AM1	96 wells plate - flat bottom

Carboxylated			
Clear	***White***	***Black***	***Description***
MT04F2-COOH	MTW04F2-COOH	MTB04F2-COOH	96 wells plate - 1x8 flat bottom wells strips on 12 x 8 frame
MG04F-COOH	MGW04F-COOH	MGB04F-COOH	96 wells plate - 1x8 flat bottom wells strips on single well holding frame
MC04F-COOH	MCW04F-COOH	MCW04F-COOH	96 wells plate - flat bottom

PCR 8 STRIP TUBES and PLATES

Streptavidin coated			
Code	***Description***	***q.ty/pack***	***q.ty/case***
PCR0STF-SA5/100	Streptavidin coated PCR tubes 8 wells	10 (sealed barrier bag)	50 or 100
PCR96010STF-SA5/100	Streptavidin coated PCR plate 96 well non-skirted	1 (sealed barrier bag)	50 or 100
PCR96020STF-SA5/100	Streptavidin coated PCR plate 96 well semi-skirted	1 (sealed barrier bag)	50 or 100
PCR96030STF-SA5/100	Streptavidin coated PCR plate 96 well skirted	1 (sealed barrier bag)	50 or 100

High Binding Streptavidin coated			
Code	***Description***	***q.ty/pack***	***q.ty/case***
PCRSTDF-SB75/100	HB Streptavidin coated PCR tubes 8 wells	10 (sealed barrier bag)	50 or 100
PCR9601STDF-SB75/100	HB Streptavidin coated PCR plate 96 well non-skirted	1 (sealed barrier bag)	50 or 100
PCR9602STDF-SB75/100	HB Streptavidin coated PCR plate 96 well semi-skirted	1 (sealed barrier bag)	50 or 100
PCR9603STDF-SB75/100	HB Streptavidin coated PCR plate 96 well skirted	1 (sealed barrier bag)	50 or 100

Protein A coated			
Code	***Description***	***q.ty/pack***	***q.ty/case***
PCR06-PA	Protein A coated PCR tubes 8 wells	10 (sealed barrier bag)	50 or 100
PCR960106-PA	Protein A coated PCR plate 96 well non-skirted	1 (sealed barrier bag)	50 or 100
PCR960206-PA	Protein A coated PCR plate 96 well semi-skirted	1 (sealed barrier bag)	50 or 100
PCR960306-PA	Protein A coated PCR plate 96 well skirted	1 (sealed barrier bag)	50 or 100

Protein A/G coated			
Code	***Description***	***q.ty/pack***	***q.ty/case***
PCR08-PA/G	Protein A/G coated PCR tubes 8 wells	10 (sealed barrier bag)	50 or 100
PCR960108-PA/G	Protein A/G coated PCR plate 96 well non-skirted	1 (sealed barrier bag)	50 or 100
PCR960208-PA/G	Protein A/G coated PCR plate 96 well semi-skirted	1 (sealed barrier bag)	50 or 100
PCR960308-PA/G	Protein A/G coated PCR plate 96 well skirted	1 (sealed barrier bag)	50 or 100

Protein G coated			
Code	***Description***	***q.ty/pack***	***q.ty/case***
PCR09-PG	Protein G coated PCR tubes 8 wells	10 (sealed barrier bag)	50 or 100
PCR960109-PG	Protein G coated PCR plate 96 well non-skirted	1 (sealed barrier bag)	50 or 100
PCR960209-PG	Protein G coated PCR plate 96 well semi-skirted	1 (sealed barrier bag)	50 or 100
PCR960309-PG	Protein G coated PCR plate 96 well skirted	1 (sealed barrier bag)	50 or 100

Maleimide coated			
Code	***Description***	***q.ty/pack***	***q.ty/case***
PCR22-MAL	Maleimide coated PCR tubes 8 wells	10 (sealed barrier bag)	50 or 100
PCR960122-MAL	Maleimide coated PCR plate 96 well non-skirted	1 (sealed barrier bag)	50 or 100
PCR960222-MAL	Maleimide coated PCR plate 96 well semi-skirted	1 (sealed barrier bag)	50 or 100
PCR960322-MAL	Maleimide coated PCR plate 96 well skirted	1 (sealed barrier bag)	50 or 100

Carboxylated			
Code	***Description***	***q.ty/pack***	***q.ty/case***
PCR04-COOH	Carboxylated PCR tubes 8 wells	50 (transparent bag)	50 or 100
PCR960104-COOH	Carboxylated PCR plate 96 well non-skirted	5 (transparent bag)	
PCR960204-COOH	Carboxylated PCR plate 96 well semi-skirted	5 (transparent bag)	
PCR960304-COOH	Carboxylated PCR plate 96 well skirted	5 (transparent bag)	
OTHER SURFACES AVAILABLE UPON REQUEST			

Caps for PCR strip tubes		
Code	***Description***	***q.ty/pack***
PCR8	Strips of 8 caps	100

12x75 mm POLYSTYRENE TUBES

COATED/ SURFACE MODIFIED 12x75 mm TUBES – 5 ml – Polystyrene			
Code	***Description***	***q.ty/pack***	***q.ty/case***
TS 12750STF-SA5/1000	Streptavidin coated	20	500-1000
TS 1275STDF-SB75/1000	High Binding Streptavidin coated	20	500-1000
OTHER SURFACES AVAILABLE UPON REQUEST			

ACCESSORIES-PACKAGING

	Code	Description	Quantity Per Case
FRAMES FOR MICROPLATES	FR2	frame 12x8	200
	FRG2	single well holding frame	200
BARRIER BAGS	AB-rs	Re-sealable. Aluminum Barrier PE + Aluminum + LLDPE external dimensions mm 135 x 210	1500
DESICCANT	Sg 5	Silica Gel 5 grams bags	1500
STRIP REMOVER	SR-8	Strip remover	1000

For more information about products please visit www.biomat.it or contact us at info@biomat.it

ENVIRONMENTAL POLICY

Biomat's environmental policy exploits the solutions addressed to reduce the environmental impact of its activity through

- Reduction of packages
- Recycling
- Easier handling

We are always developing new solutions for:

- a better protection with a reduced weight of the package
- a reduced handling time
- less waste of packaging material for its customers

The current package foresees easy-handling cartons and sealed bags for the final package of the products.
Labels on package and codes on the products facilitate the identification of the type of product and of the lots.
Online Quality Certificates of products help easy traceability and reduce the waste of paper
Since 2004 Biomat adopted a recycling policy with its suppliers reducing the waste of packages of raw materials.

QUALITY

- Biomat is certified ISO 9001 since 2002
- Every lot is tested for **uniformity** and **reproducibility**
- The QC certificates are available both online and in paper format.

Our QC protocols are based on a systematic control on every lot and on the comparison with other ones, also at different stages of aging, in order to ensure the uniformity of the performance of our products under every condition.

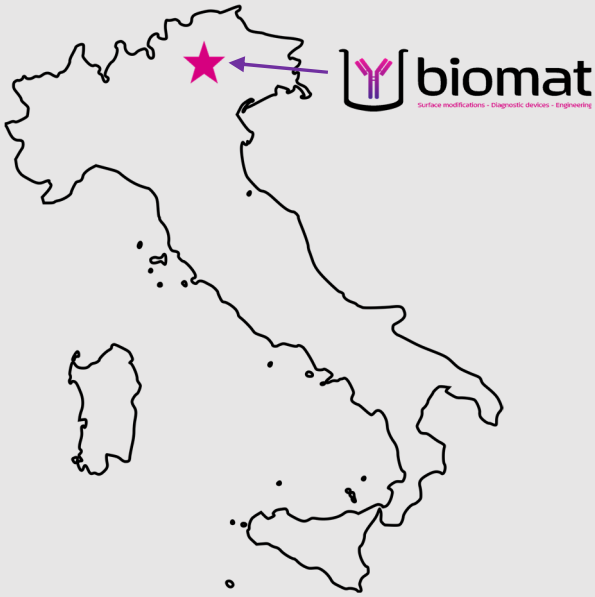
MARKETS

Biomat operates worldwide.
More that 70% of our products and services are exported to Europe, Americas, Middle East, Asia and Australia.

Through the network of our distributors and the assistance of our headquarter we warrant short time deliveries and competent technical assistance.

NOTES

biomat
Surface modifications - Diagnostic devices - Engineering



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