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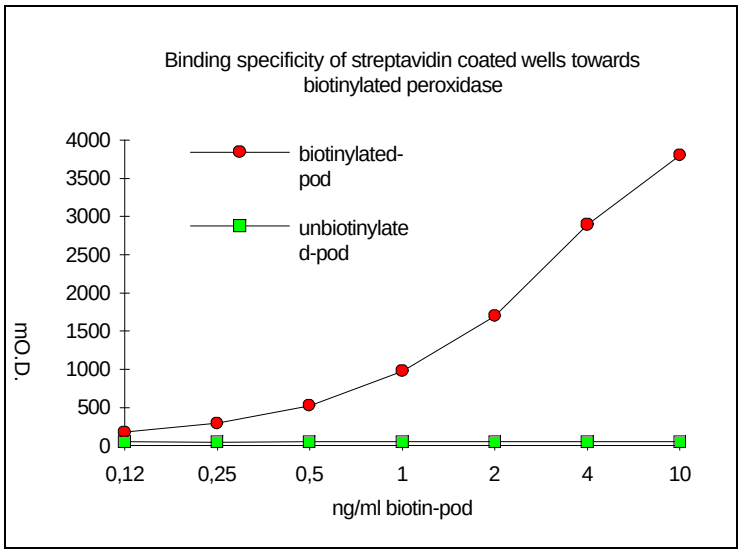
STREPTAVIDIN COATED SURFACES

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. 60.000) with very high affinity for biotin (Ka=10 $^{15}M^{-1}$); the bond is the strongest known non-covalent biological interaction.



Biotin is a small molecule which can be conjugated to many proteins without loosing 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.

TECHNICAL NOTES 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.

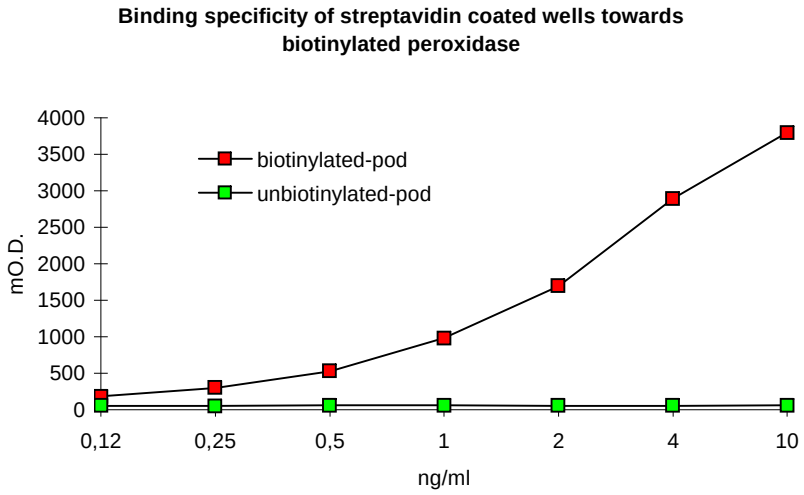
results	**12 pmol/ well (200 µl volume)**

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' RT

After a washing step, the wells were incubated with TMB and blocked with sulphuric acid 1N

The OD values were read at 450 nm

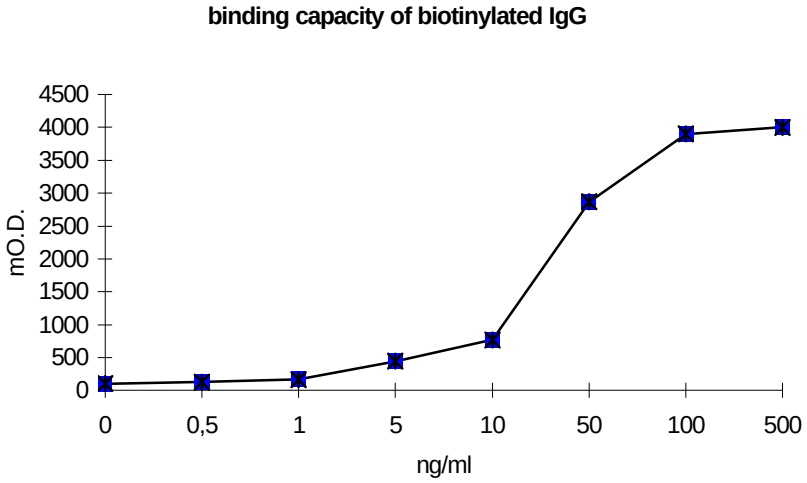


3. binding capacity towards biotinylated IgG

Streptavidin coated wells were incubated with solutions (from 500 to 0 ng/ml)of biotinylated IgG for 30' RT

After a washing step, the wells were incubated with AHIgG-Pod for 30' RT, again washed and incubated with TMB and blocked with sulphuric acid 1N

The OD values were read at 450 nm



4. Uniformity of biotin binding

Test conditions:

- A 96 wells plate was incubated with a biotinylated peroxidase solution.
- 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%

specificity	**CV% < 5**

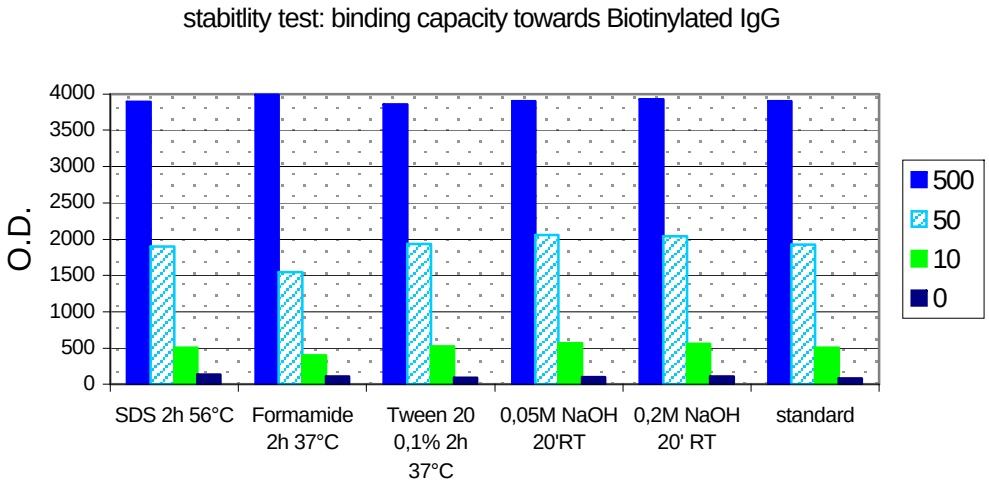
5. Stability tests:

5.1. Endurance under strong chemical contacts

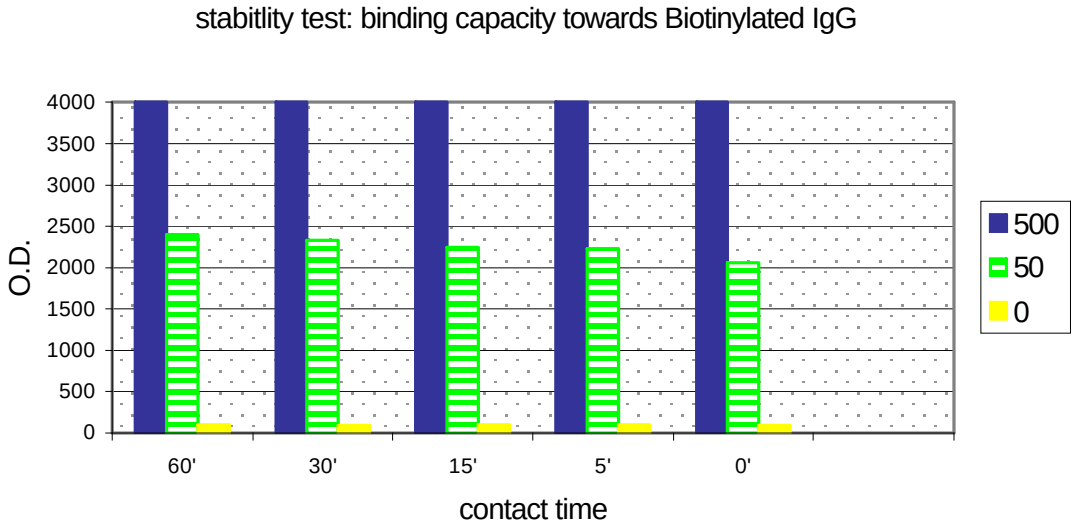
Endurance under strong chemical contacts was determined following method 1 (Biotinylated IgG from 500 to 0 ng/ml) where the first washing step was substituted by the following incubations

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	RT 20'
0.2M NaOH	RT 20'
standard -0.1% Tween 20 in 0.1M PBS	4 x
Urea 8M	60'-30'-15'-5'

Results



Urea 8M



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**
OD	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.

The results show the exceptional stability of the coating .

temperature	**4 ° C**	**RT**
OD	1890	1950
CV%	1.8	2.3

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	3 days
	22°-23° C	12 h.
	- 20° C	10 h.
	37° C	3 days
	22°-23° C	3 days

results