NEUTRAVIDIN® COATED SURFACE

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. 60.000) 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.

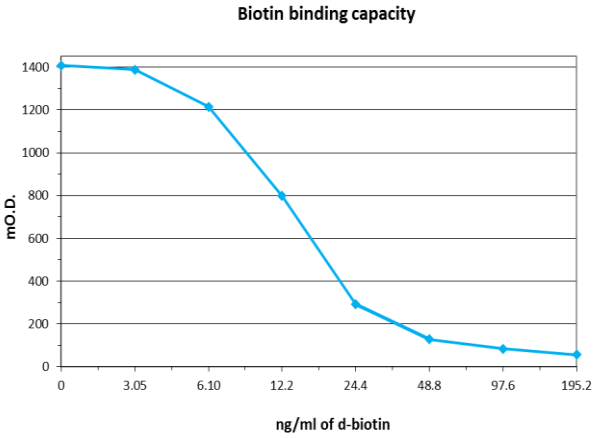
TECHNICAL NOTE N. 32

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

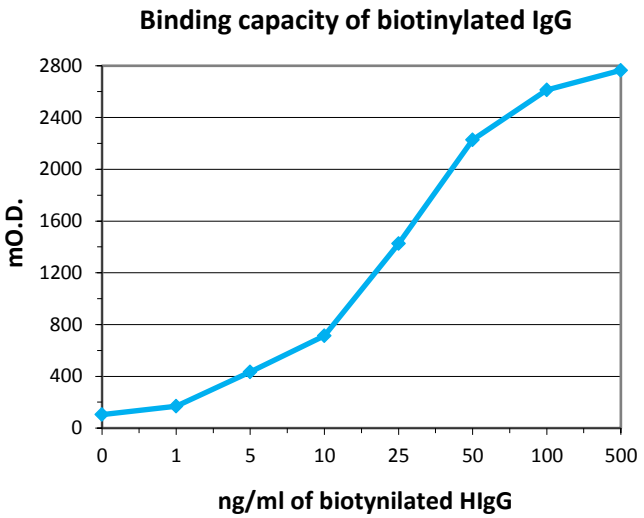
Neutravidin coated wells were incubated with biotin solutions (from 0 to 195.2 ng/ml) containing 2 ng/ml of biotinylated peroxidase for 30' R.T.
After a washing step, the wells were incubated with TMB and blocked with sulphuric acid 1N.
The OD values were read at 450 nm.



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' R.T.
After a washing step, the wells were incubated with AHIgG-HRP for 30' RT, 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' R.T.
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.