STREPTAVIDIN COATED SURFACE

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

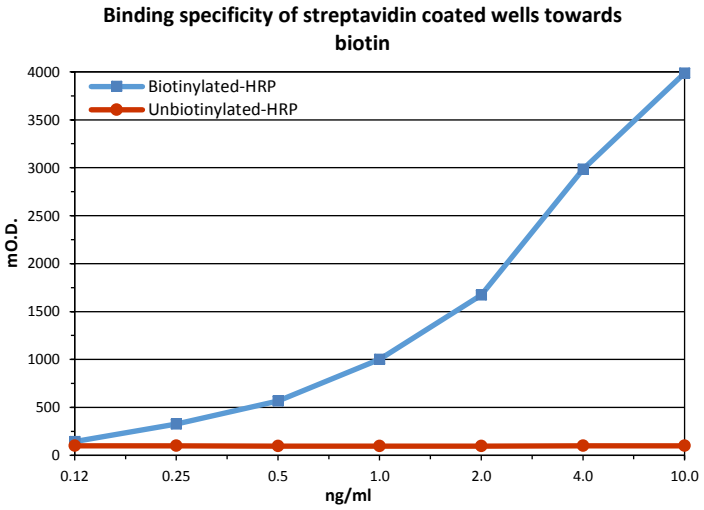
1. Binding capacity towards biotin

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)

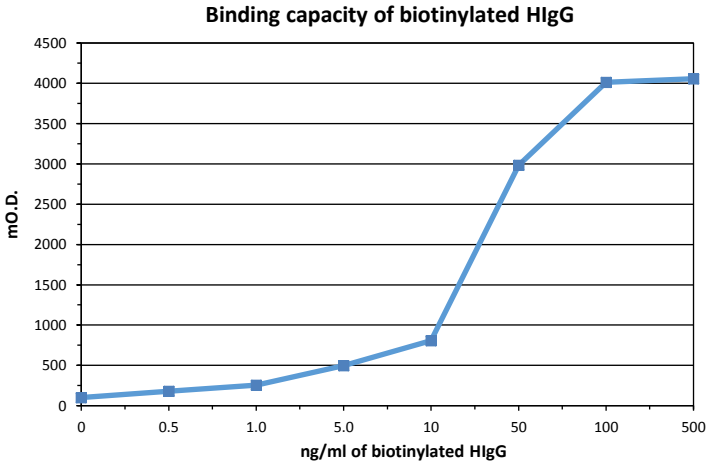
2. Specificity towards biotin

Streptavidin coated wells were incubated with solutions (from 10 to 0.12 ng/ml) of biotinylated peroxidase and unbiotinylated peroxidase (blanks) for 30' 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.



3. Binding capacity towards biotinylated HIgG

Streptavidin 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-Pod for 30' R.T., 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)