HIGH BINDING CAPACITY SURFACE FOR IMMUNOLOGICAL ASSAYS

TECHNICAL NOTE N. 10

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.