

## PROTEIN A COATED SURFACE

### TECHNICAL NOTE N. 24

#### Sensitivity test

1. Add 100  $\mu$ l 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<sup>®</sup> four times
3. Add 100  $\mu$ l /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<sup>®</sup> four times
5. Add 100  $\mu$ l /well of TMB substrate solution and incubate 15 minutes at room temperature
6. Stop the substrate reaction by adding 100  $\mu$ l /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

