

## JACALIN COATED SURFACE

### TECHNICAL NOTE N. 29

#### Sensitivity test

1. Add 100  $\mu$ l of different concentrations of biotinylated human IgA (from 0.05 to 10  $\mu$ g/ml) to the wells of Jacalin coated plate diluted in pure distilled water containing 1 mM  $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$  + 1 mM  $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$  and incubate for 60 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 at 1 mg/ml), diluted 1:20.000 in pure distilled water containing 1 mM  $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$  + 1 mM  $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$ ) 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 5 minutes at room temperature
6. Stop the substrate reaction by adding 100  $\mu$ l /well of sulphuric acid 0.3 N and read the optical density values at 450 nm

The microplate sensitivity was calculated as the lowest biotinylated IgA concentration higher than the mean optical density plus 5 S.D. of 0  $\mu$ g/ml biotinylated IgA concentration.

Our experiment gave the following results:

- 0  $\mu$ g/ml biotinylated IgA optical density mean (coming from 4 replicates) = 0.0655
- standard deviation = 0.0050
- mean + 5 S.D. = 0.0905
- sensitivity = 0.0161  $\mu$ g/ml (1.61 ng/well) of biotinylated human IgA

Figure 2

