

## JACALIN COATED SURFACE

### TECHNICAL NOTE N. 28

#### Binding capacity 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  $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$ . 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 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
3. Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween 20<sup>®</sup> four times
4. Add 100  $\mu$ l /well of TMB substrate solution and incubate 5 minutes at room temperature
5. 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 data show that a plateau has got starting with a biotinylated human IgA concentration of 6  $\mu$ g/ml.

This concentration means the well binding capacity we can express as:

$\mu$ g/well = 0.6 (600 ng/well)

pmol/well= 4.0 (this result is calculated considering the IgA1 M.W. = 150.000)

Figure 1

