## JACALIN COATED SURFACE

## **TECHNICAL NOTE N. 28**

## **Binding capacity test**

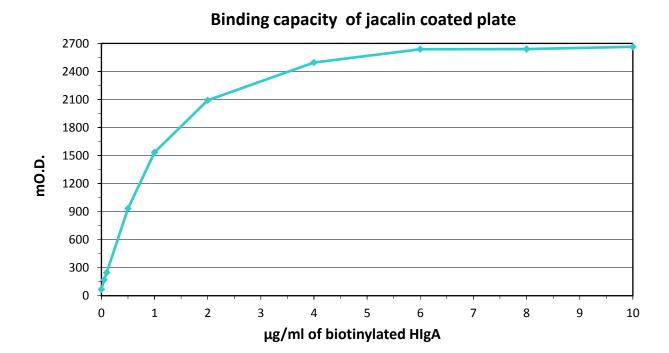
- 1. Add 100  $\mu$ l of different concentrations of biotinylated human IgA (from 0.05 to 10  $\mu$ g/ml) to the wells of Jacalin coated plate diluted in pure distilled water containing 1 mM CaCl<sub>2</sub> 2 H<sub>2</sub>O + 1 mM MnCl<sub>2</sub> 4 H<sub>2</sub>O. Incubate for 60 minutes at room temperature
- Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween 20<sup>®</sup> four times Add 100 μl /well of Streptavidin-HRP (BioSpa product code SB01-61 at 1 mg/ml), diluted 1:20.000 in pure distilled water containing 1 mM CaCl<sub>2</sub> • 2 H<sub>2</sub>O + 1 mM MnCl<sub>2</sub> • 4 H<sub>2</sub>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  $20^{\text{(R)}}$  four times
- 4. Add 100 µl /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  $\mu\text{g}/\text{ml}.$ 

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