

## GOAT ANTI-HUMAN IgG COATED SURFACE

### TECHNICAL NOTE N. 52

#### Binding capacity and sensitivity test

1. Add 100  $\mu$ l of different concentrations of human IgG (Jackson ImmunoResearch code 009-000-003 from 0.01 to 2  $\mu$ g/ml) to the wells of goat anti human IgG coated plate 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<sup>®</sup> 20 three times;
3. Add 100  $\mu$ l /well of Goat anti-human IgG (H+ L)-HRP (Jackson ImmunoResearch code 115-035-003, 0.8 mg/ml, diluted 1:50,000) 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<sup>®</sup> 20 three 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 0.3 N and read the optical density values at 450 nm.

The data show that a plateau has got starting with a human IgG concentration of 0.25  $\mu$ g/ml. This concentration means the well binding capacity we can express as:

- $\mu$ g/well = 0.25 (25 ng/well)

The microplate sensitivity was calculated as the lowest human IgG concentration higher than the mean optical density plus 5 S.D. of 0  $\mu$ g/ml human IgG concentration. Our experiment gave the following results:

- 0  $\mu$ g/ml human IgG optical density mean (coming from 8 replicates) = 0.093
- standard deviation = 0.005
- mean + 5 S.D. = 0.118
- sensitivity = 0.001  $\mu$ g/well of human IgG

Figure 1

