

GOAT ANTI-HUMAN IGG COATED SURFACE

TECHNICAL NOTE N. 52

Binding capacity and sensitivity test

- 1. Add 100 μl of different concentrations of human IgG (Jackson ImmunoResearch code 009-000-003 from 0.01 to 2 μg/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 $^{\circledR}$ 20 three times;
- 3. Add 100 μl /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[®] 20 three times;
- 5. Add 100 µl /well of TMB substrate solution and incubate 15 minutes at room temperature;
- 6. Stop the substrate reaction by adding 100 μ 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 μ g/ml. This concentration means the well binding capacity we can express as:

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

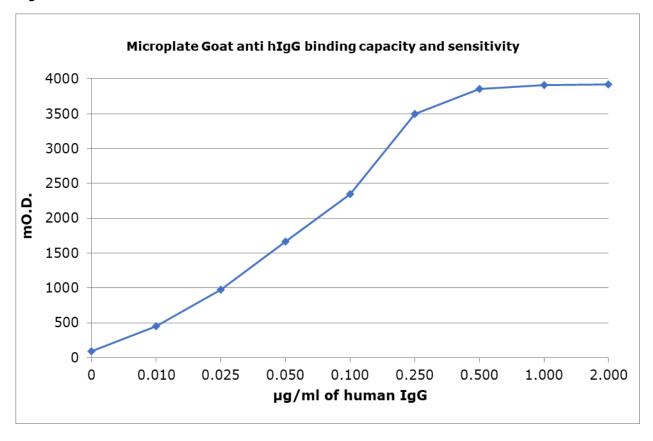
The microplate sensitivity was calculated as the lowest human IgG concentration higher than the mean optical density plus 5 S.D. of 0 μ g/ml human IgG concentration. Our experiment gave the following results:

- 0 μg/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

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Figure 1



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