GOAT ANTI-HUMAN IgM COATED SURFACE

TECHNICAL NOTE N. 54

Binding capacity and sensitivity test

1. Add 100 µl of different concentrations of human IgM (Jackson ImmunoResearch code 009-000-012 from 0.1 to 4 µg/ml) to the wells of goat anti human IgM 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® 20 three times;

3. Add 100 µl /well of Goat anti-human IgM (H+ L)-HRP (Jackson ImmunoResearch code 115-035-043, 0.8 mg/ml, diluted 1:25,000) 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 µl /well of sulfuric acid 0.3 N and read the optical density values at 450 nm.

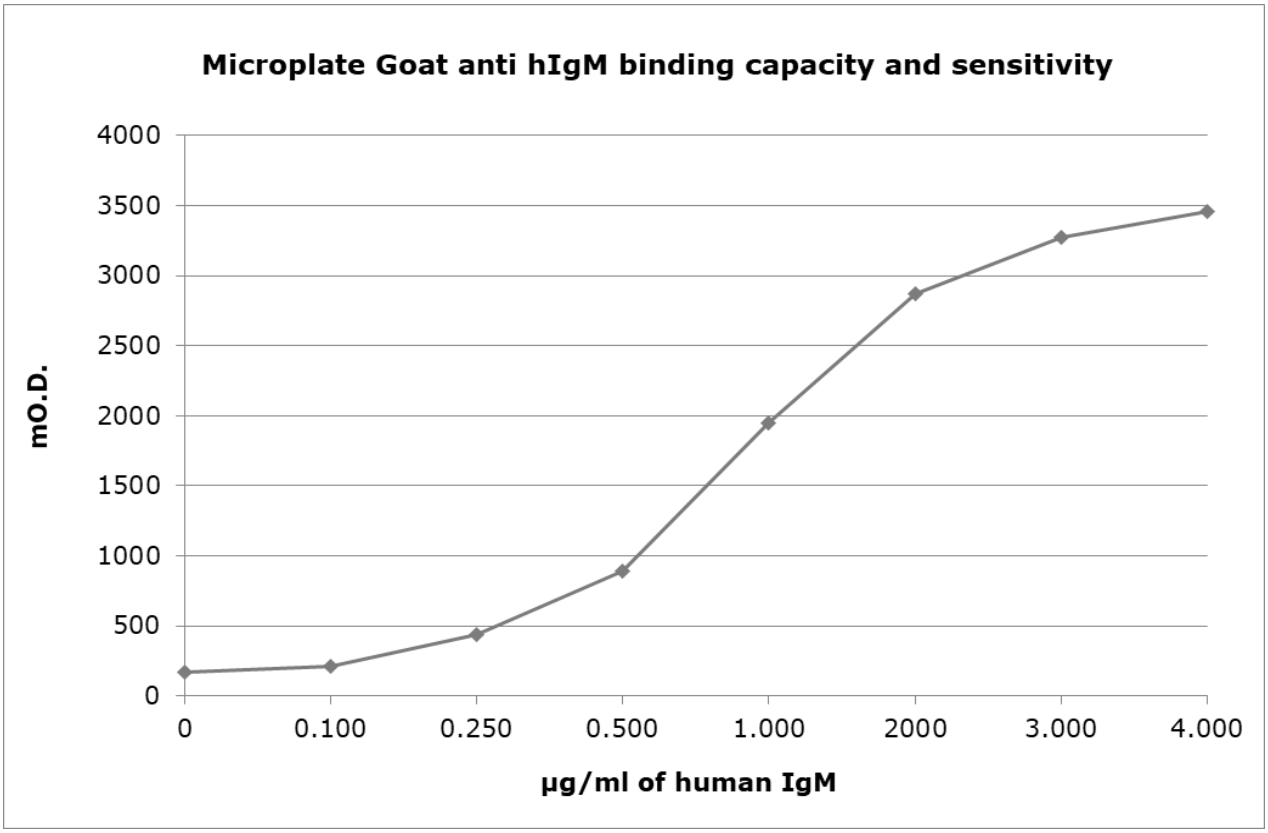
The data show that a plateau has got starting with a human IgM concentration of 3.00 µg/ml.
This concentration means the well binding capacity we can express as:

- µg/well = 0.300 (300 ng/well)

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

- 0 µg/ml human IgM optical density mean (coming from 8 replicates) = 0.168
- standard deviation = 0.009
- mean + 5 S.D. = 0.213
- sensitivity = 0.01 µg/well of human IgM

Figure 1


Microplate Goat anti hIgM binding capacity and sensitivity
mO.D.
4000
3500
3000
2500
2000
1500
1000
500
0
0
0.100
0.250
0.500
1.000
2000
3.000
4.000
µg/ml of human IgM