

GOAT ANTI-HUMAN IgM COATED SURFACE

TECHNICAL NOTE N. 54

Binding capacity and sensitivity test

1. Add 100 μ l of different concentrations of human IgM (Jackson ImmunoResearch code 009-000-012 from 0.1 to 4 μ g/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 μ l /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 μ l /well of TMB substrate solution and incubate 15 minutes at room temperature;
6. Stop the substrate reaction by adding 100 μ l /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 μ g/ml. This concentration means the well binding capacity we can express as:

- μ g/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 μ g/ml human IgM concentration. Our experiment gave the following results:

- 0 μ g/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 μ g/well of human IgM

Figure 1

