SECONDARY ANTIBODIES COATED SURFACE: GOAT ANTI MOUSE IgG Fcγ (Subclasses 1+2a+2b+3)

TECHNICAL NOTE N. 37

Binding capacity and sensitivity test

1. Add 100 µl of different concentrations of mouse IgG (from 0.025 to 4 µg/ml) to the wells of goat anti mouse 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® 20 four times
3. Add 100 µl /well of Goat anti-mouse IgG (H+ L)-HRP (Jackson ImmunoResearch code 115-035-003, diluted 1: 150.000) 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 four 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 sulphuric acid 0.3 N and read the optical density values at 450 nm

The data show that a plateau has got starting with an IgG mouse concentration of 1.0µg/ml.

This concentration means the well binding capacity we can express as:

- µg/well = 0.1 (100 ng/well)
- pmol/well= 0.625 (this result is calculated considering the IgG M.W. = 160.000)

The microplate sensitivity was calculated as the lowest mouse IgG concentration higher than the mean optical density plus 5 S.D. of 0 µg/ml mouse IgG concentration.

Our experiment gave the following results:

- 0 µg/ml mouse IgG optical density mean (coming from 8 replicates) = 0.108
- standard deviation = 0.014
- mean + 5 S.D. = 0.178
- sensitivity = 0.012µg/well of mouse IgG

Figure 1