SECONDARY ANTIBODIES COATED SURFACE: GOAT ANTI RABBIT IgG Fc

TECHNICAL NOTE N. 38

Binding capacity and sensitivity test

1. Add 100 µl of different concentrations of rabbit IgG (from 0.025 to 4 µg/ml) to the wells of goat anti rabbit 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-rabbit IgG (H+ L)-HRP (Jackson ImmunoResearch code 111-035-003, 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 1 N and read the optical density values at 450 nm

The data show that a plateau has got starting with an IgG rabbit concentration of 1.0 µg/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 Da)

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

Our experiment gave the following results:

- 0 µg/ml rabbit IgG optical density mean (coming from 8 replicates) = 0.121
- standard deviation = 0.013
- mean + 5 S.D. = 0.186
- sensitivity = 0.010µg/well of rabbit IgG

Figure 1