

PROTEIN A COATED SURFACE

TECHNICAL NOTE N. 24

Sensitivity test

- 1. Add 100 µl of different concentrations of human biotinylated IgG (from 1.56 to 100 ng/ml) to the wells of Protein A coated plate and incubate for 30 minutes at room temperature
- 2. Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween 20^{\circledR} four times
- 3. Add 100 μ l /well of Streptavidin-HRP (BioSpa product code SB01-61, diluted 1:20.000) 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 μ l /well of sulphuric acid 1 N and read the optical density values at 450 nm

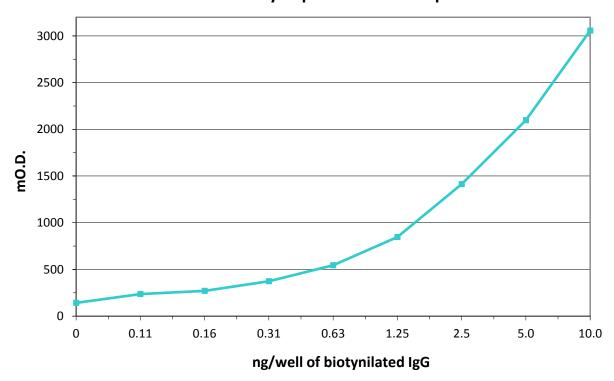
The microplate sensitivity was calculated as the lowest biotinylated IgG concentration higher than the mean optical density plus 5 S.D. of 0 ng/ml biotinylated IgG concentration.

Our experiment gave the following results:

- 0 ng/ml biotinylated IgG optical density mean (coming from 8 replicates) = 0.141
- standard deviation = 0.019
- mean + 5 S.D. = 0.236
- sensitivity = 0.113 ng/well of human IgG

Figure 2

Sensitivity of protein A coated plate



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