

PROTEIN G COATED SURFACE

TECHNICAL NOTE N. 26

Binding capacity test

- 1. Add 100 μ l of different concentrations of biotinylated human IgG, (diluted from 0.25 to 8.0 μ g/ml) to the wells of Protein G 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 (Biomat code 200-3) three times
- 3. Add 100 μ l/well of Streptavidin-HRP diluted 1:30,000 mixed with Streptavidin at 5 μ g/ml and incubate for 30 minutes at RT
- 4. Empty the wells and wash with 0.1 M PBS pH 7.2+0.05% Tween[®] 20 (Biomat code 200-3) three times
- 5. Add 100 μ l /well of TMB substrate solution (Biomat code 500-1) and incubate 15 minutes at room temperature
- 6. Stop the substrate reaction by adding 100 μ l/well of sulphuric acid 1 N (Biomat code 600-1) and read the optical density values at 450 nm

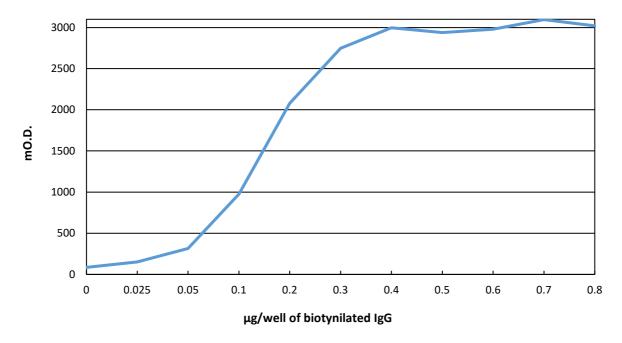
The data show that a plateau has got starting with a biotinylated human IgG concentration falling between 4.0 and 5.0 µg/ml.

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

- $-\mu g/well = 0.4-0.5 (400-500 ng/well)$
- pmol/well= 2.66-3.33 (this result is calculated considering the IgG M.W. = 150 kDa)

Figure 1

Binding capacity of protein G coated plate



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