PROTEIN A COATED SURFACE

TECHNICAL NOTES N. 23

1. Add 100 µl of different concentrations of biotinylated human IgG, (diluted from 0.25 to 8.0 µg/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 (Biomat code 200-3) three times
3. Add 100 µl/well of Streptavidin-HRP diluted 1:30,000 mixed with Streptavidin at 5 µg/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 µl /well of TMB substrate solution (Biomat code 500-1) and incubate 15 minutes at room temperature
6. Stop the substrate reaction by adding 100 µl/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:

- µg/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