

MALEIMIDE COATED SURFACE

TECHNICAL NOTE N. 35

Binding capacity test

1. Add 100 μl of different concentrations of Glutathione Ethyl Ester Biotin Amide (BioGEE) from 0.1 to 10 $\mu\text{g}/\text{ml}$ diluted in 10 mM PBS pH 6.6, 115 mM NaCl, 100 mM EDTA, 40 mM sucrose and incubate for 2 hours at room temperature
2. Empty the wells and wash with ELISA wash buffer (Biomat code 200-3) four times
3. Add 100 $\mu\text{l}/\text{well}$ of Streptavidin-HRP (BioSpa product code SB01-61 at 1 mg/ml), diluted 1:5,000 and incubate for 45 minutes at room temperature
4. Empty the wells and wash with ELISA wash buffer (Biomat code 200-3) four times
5. Add 100 $\mu\text{l}/\text{well}$ of TMB substrate solution (Biomat code 500-1) and incubate 10-15 minutes at room temperature
6. Stop the substrate reaction by adding 100 $\mu\text{l}/\text{well}$ of Stop solution (Biomat code 600-1) and read the optical density values at 450 nm

The data show that a plateau has got starting with a BioGEE concentration of 2.5 $\mu\text{g}/\text{ml}$.

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

- $\mu\text{g}/\text{well} = 0.25 \mu\text{g}$ (250 ng/well)
- $\text{pMol}/\text{well} = 440$ (this result is calculated considering the BioGEE M.W. = 561 Da)

Figure 1

