MAB ANTI GST (Glutathione S-Transferase) COATED SURFACE

TECHNICAL NOTES N. 44 – binding capacity and sensitivity test

1. Prepare a standard curve of purified recombinant GST (*GenScript* code Z02039-1), from 0.01 to 4.0 µg/ml, diluted in Sample Diluent (*Biomat* code 400-1*)*;
2. Add 100 µl of different concentrations of purified recombinant GST to the wells of monoclonal mouse anti-GST coated plate and incubate for 60 minutes at room temperature;
3. Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* three times;
4. Add 100 µl/well of Goat anti-GST-HRP (*GenScript* code A01380), diluted 1:4,000 in Diluent for HRP conjugate (*Biomat* code 400-2) and incubate for 60 minutes at room temperature;
5. Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* three times;
6. Add 100 µl/well of TMB substrate solution (*Biomat* code 500-1) and incubate 15 minutes at room temperature;
7. Stop the substrate reaction by adding 100 µl/well of sulphuric acid (*Biomat* code 600-1) and read the optical density values at 450 nm

The data show that a plateau has got starting with a GST 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= 3.4 (this result is calculated considering the GST M.W. = 29,000 Da)

The microplate sensitivity was calculated as the lowest GST concentration higher than the mean optical density plus 5 S.D. of 0 µg/ml GST concentration.
Our experiment gave the following results:

- 0 µg/ml GST optical density mean (coming from 8 replicates) = 0.144
- standard deviation = 0.017
- mean + 5 S.D. = 0.085
- sensitivity = 1.2 ng/well of GST