MAB ANTI HIS (Polyhistidine)-tag COATED SURFACES

TECHNICAL NOTES N. 46 – binding capacity and sensitivity test

1. Prepare a standard curve of purified recombinant HSA His tagged (*AcroBiosystems* code HSA-H5220), from 0 to 4.0 µg/ml, diluted in Phosphate buffer pH 7.2 (*Biomat* code 100-1) + 0.25% Tween®;
2. Add 100 µl of different concentrations of purified recombinant HSA His tagged to the wells of monoclonal mouse anti-His Tag 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 rabbit anti-HSA-HRP (*Immunechem* code ICP0101 1 mg/ml), diluted 1:20,000 in Phosphate buffer pH 7.2 (*Biomat* code 100-1) + 0.25% Tween® 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 an HSA His tagged concentration including between 2 and 4 µg/ml. This concentration means the well binding capacity we can express as:

- µg/well = 0.2 – 0.4 (200 – 400 ng/well)
- pMol/well= 3 – 6 (this result is calculated considering the HSA His tagged M.W. = 67.3 kDa)

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

- 0 µg/ml HSA His tagged optical density mean (coming from 8 replicates) = 0.140
- standard deviation = 0.028
- mean + 5 S.D. = 0.140
- sensitivity = 5 ng/well of HSA His tagged