

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 μ g/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 μ l/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 μ l/well of sulphuric acid (*Biomat* code 600-1) and read the optical density values at 450 nm.

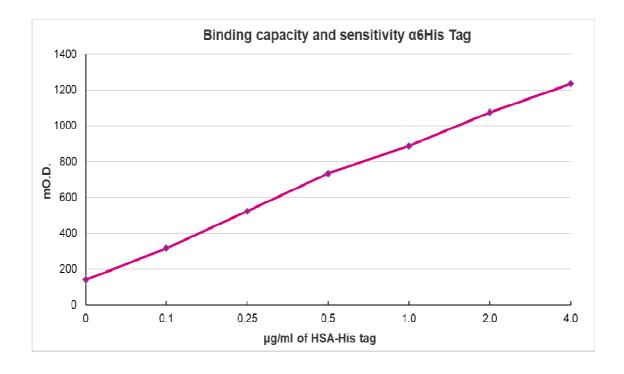
The data show that a plateau has got starting with an HSA His tagged concentration including between 2 and 4 μ g/ml. This concentration means the well binding capacity we can express as:

- μ g/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 μ g/ml HSA His tagged concentration.

Our experiment gave the following results:

- $0 \mu g/ml$ 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



www.biomat.it 1