MAB ANTI DYKDDDDK-tag COATED SURFACES

TECHNICAL NOTES N. 49 – binding capacity and sensitivity test

1. Prepare a standard curve of purified Recombinant Human Flag Ubiquitin (*BostonBiochem* code #U-120), from 0.25 to 6.0 µg/ml, diluted in Sample Diluent (*Biomat* code 400-1);
2. Add 100 µl of different concentrations of purified Recombinant Human Flag Ubiquitin to the wells of monoclonal mouse anti-DYKDDDDK coated plate and incubate for 2 h at 37°C;
3. Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* three times;
4. Add 100 µl/well of Mab anti-Ubiquitin-biotin (*BioLegend* code 646305) 0.25 µg/ml diluted in Sample Diluent (*Biomat* code 400-1) 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 Streptavidin-Peroxidase (*BioSpa* 1mg/ml code SB01-61), diluted 1: 20,000 in Diluent for HRP conjugate (*Biomat* code 400-2) and incubate for 30 minutes at room temperature;
7. Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* three times;
8. Add 100 µl/well of TMB substrate solution (*Biomat* code 500-1) and incubate 15 minutes at room temperature;
9. 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 Recombinant Human Flag Ubiquitin concentration close to 6 µg/ml.
This concentration means the well binding capacity we can express as:

- µg/well = ~ 0.6 (600 ng/well)
- pMol/well= ~60 (this result is calculated considering the Recombinant Human Flag Ubiquitin M.W. = 9.8 kDa)

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

- 0 µg/ml Ubiquitin optical density mean (coming from 8 replicates) = 0.174
- standard deviation = 0.009
- mean + 5 S.D. = 0.045
- sensitivity = 12 ng/well of Ubiquitin