MAB ANTI HIS (Polyhistidine)-tag COATED SURFACES

The Biomat product is a 96 well coated microplate with mouse monoclonal anti His-tag and a non-proteic buffer to block non-specific binding sites and to maintain stable activity.
These plates are designed to specifically bind Histidine tagged proteins with His tag located at N-terminus or C-terminus.

Features of mouse monoclonal anti-HIS antibody coated plates:

- ideal to bind proteins with HIS (Poly histidine) tag
- pre-purification of cell lysates is not necessary before screening and analysis of recombinant HIS-tagged protein expression by ELISA using the plates
- after immobilizing HIS fusion proteins, the plates are useful for screening for sera antibodies to the fusion protein
- it can be applied for analysis of different length of His tag including: hexa-His (6 His), penta-His (5 His) and tetra-His (4 His)

Product specifications

Available configurations
96-well microplates with 12 removable 8-well strips.

Coating
Mouse monoclonal anti-HIS is coated using 100 µl/well. The strips are post-coated (blocked) for low non specific binding and long-term stability with non proteic blocking buffer.

Binding capacity[1]
Microplate was saturated with HSA-His Tag to a concentration between 2.0 and 4.0 µg/ml (200 – 400 ng/well) in an ELISA format using rabbit anti HSA-HRP as detector and TMB as substrate (see technical note no. 46 for data and experiment details).
The Biomat Mouse Monoclonal anti-HIS microplate shows a nominal **binding capacity between 200 - 400 ng/well of HAS-His Tag**

Sensitivity[1]
HSA-His tag was detected at a concentration significantly above background in an ELISA format using rabbit anti HSA-HRP as detector and TMB as substrate (see technical note no. 46 for data and experiment details).
The Biomat Mouse Monoclonal anti-HIS microplate shows a **sensitivity of ~ 5 ng/well of HSA-His Tag**

Uniformity
Microplates show a **CV% less than 6** when used as a sandwich of HSA-His Tag in an ELISA format using rabbit anti HSA-HRP as detector and TMB as substrate.

Reagent Compatibility
Some reagents may interfere with the test results. Check the reagents concentration in sample according to the reagent compatibility tests table. Dialyse or dilute samples if needed.

Substance	Compatible Concentration
Triton X-100	≤ 2%
Tween 20	≤ 1%
EDTA	≤ 20 mM
β-ME	≤ 10 mM
Urea	≤ 1 M
Guanidine HCl	≤ 125 mM
Glycerol	≤ 1%
Deoxycholic Acid	≤ 1%
Imidazole	≤ 62.5 mM
SDS	**DO NOT** use any reagent that contains this reagent in the loading and wash buffer, since it will denature the anti-His antibody and destroy its ability to bind His tagged proteins

Storage and Stability
The microplates, under the indicated storage conditions 2-8 °C, are stable until the expiration date printed on the label.
If opened, store in closed pouch with desiccant and use within the expiration date.

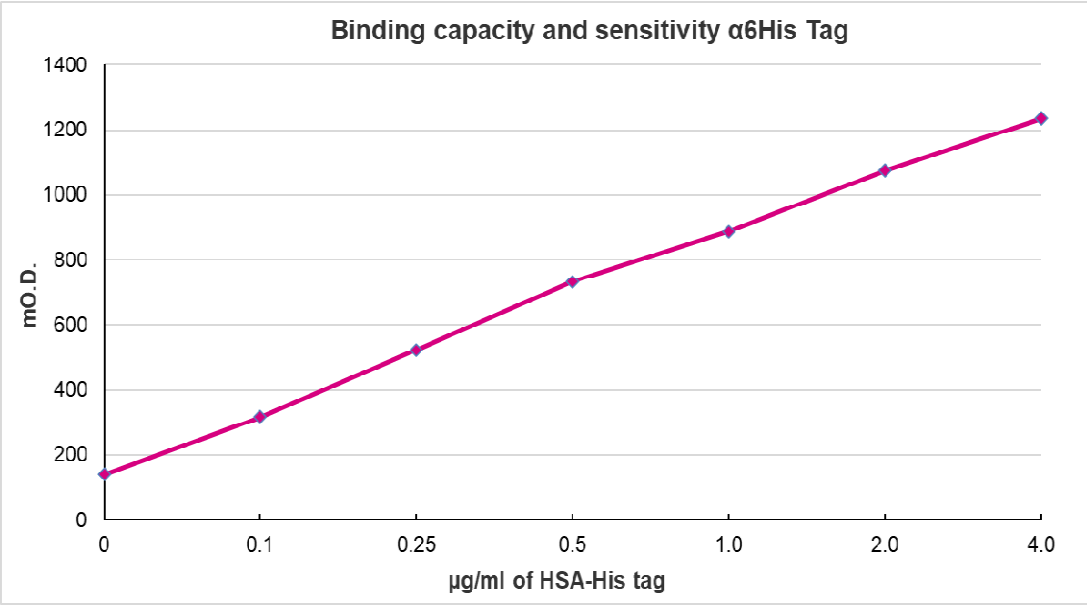
Note[1]: The binding capacity and sensitivity varies, depending on protein size and structure. Generally, proteins with low molecular weight (M.W.) can be more sensitive and more bounded to His plate than ones with higher M.W.

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



TECHNICAL NOTES N. 47 – General ELISA procedures using anti-HIS tag coated plates

Note: The following procedures use as revealing system a conjugate HRP labelled and TMB as substrate/chromogen. It is however possible to use other enzymatic tracers with their appropriate substrate/chromogen.

Procedure 1

This procedure is useful to perform protein expression screening in samples; the operator needs the availability of a negative and positive control test sample, containing His-tagged protein, and a polyclonal HRP-conjugated antibody against target protein

1) Add 100 µl of test samples, negative control and positive control into anti-His tag wells and incubate for 60 minutes at room temperature
2) Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* four times
3) Add 100 µl/well of a **polyclonal** HRP anti-target protein and incubate for 60 minutes at room temperature
4) Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* four times
5) Add 100 µl/well of TMB substrate solution (*Biomat* code 500-1) and incubate 15 minutes at room temperature
6) Stop the substrate reaction by adding 100 µl/well of of sulphuric acid (*Biomat* code 600-1) and read the optical density values at 450 nm
7) Calculation of results
   The obtained optical density values of samples are evaluated against the optical density values of the negative and positive controls.

Procedure 2

This procedure is useful for to quantify His-tagged proteins in samples.
Before test, the operator should do preliminary experiments to set up a standard curve of His-tagged protein of interest. Moreover, it is necessary the use of a polyclonal HRP-conjugated antibody against target protein.

1) Add 100 µl of test samples and standard curve points into anti-His tag wells and incubate for 60 minutes at room temperature
2) Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* four times
3) Add 100 µl/well of a **polyclonal** HRP anti-target protein and incubate for 30 minutes at room temperature
4) Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* four times
5) Add 100 µl/well of TMB substrate solution (*Biomat* code 500-1) and incubate 15 minutes at room temperature
6) Stop the substrate reaction by adding 100 µl/well of of sulphuric acid (*Biomat* code 600-1) and read the optical density values at 450 nm
7) Calculation of results

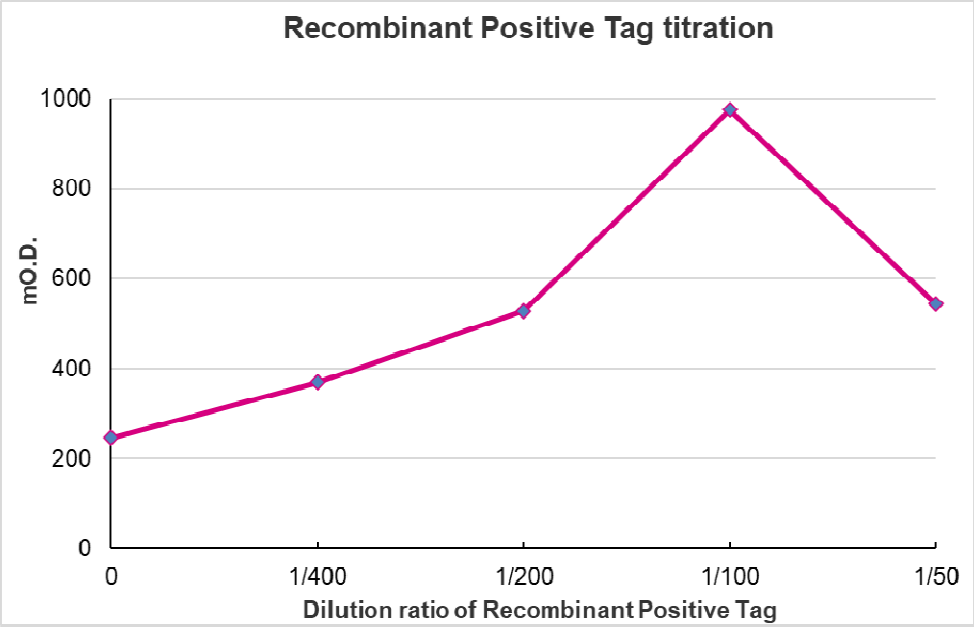
   The obtained optical density values of the standards (y-axis, linear) are plotted against their concentration (x-axis, linear) on graph paper or using an automated method. A good fit is provided with point-to-point curve, because this method gives the highest accuracy in data calculation.
   The concentration of the samples can be read directly from the curve.
   If the sample optical density value is higher than the upper limit of the standard curve, the sample should be diluted and the experiment rerun.

TECHNICAL NOTES N. 48 – Evaluation of Recombinant Posi-Tag Epitope Tag Protein (*BioLegend* catalog 931301) using anti-HIS tag coated plates; it comes in a buffer containing SDS, but with unknown concentration.

1) Dilute the Recombinant Posi-Tag Epitope Tag Protein 1:50, 1:100, 1:200, 1:400 in Sample Diluent (*Biomat* code 400-1) and add 50 µl of each dilution into anti-His tag wells and incubate for 60 minutes at room temperature
2) Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* four times
3) Add 50 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
4) Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* four times
5) Add 100 µl/well of TMB substrate solution (*Biomat* code 500-1) and incubate 15 minutes at room temperature
6) Stop the substrate reaction by adding 100 µl/well of of sulphuric acid (*Biomat* code 600-1) and read the optical density values at 450 nm
7) Evaluation of results

   The obtained optical density values of the dilutions of the Recombinant Positive Tag control (y-axis, linear) are plotted against their concentration, here reported as a dilution ratio (x-axis, linear) on graph paper. A good fit is provided with point-to-point curve, because this method gives the highest accuracy in data calculation.

   The figure below shows the titration curves obtained with the Mab anti 6xHis.



Comment on the data obtained

First of all, it is worth to pointing out that the Recombinant Posit-Tag Epitope Protein lysate contains the following proteins, fused in the following order: GST, T7, HSV, c-myc, VSV, Glu-Glu, V5, E-tag, DYKDDDDK, S-tag, HA, and 6-His. The predicted M.W. = ~ 45 kDa

As a first observation to the data obtained it is important to underline the specific link between the Mab anti 6xHis plate and the Recombinant Posit-Tag Epitope Protein.

Another important observation to point out is the interference of the SDS concentration in the Recombinant Posit-Tag Epitope Protein lysate. In fact, the 1:50 dilution results in O.D. less than the 1:100 dilution due to the higher SDS concentration present in the dilution. With the dilution 1:100 and following the interference from SDS it is proportional to its concentration and the dilution test appears to work.

The Recombinant Posi-Tag Epitope Protein lysate demonstrates it can be used in tandem with a polyclonal anti GST-HRP tracer to qualify the Mab anti 6xHis plate.