MAB ANTI DYKDDDDK-tag COATED SURFACES

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

Features of mouse monoclonal anti- DYKDDDDK antibody coated plates:

- ideal to bind proteins with DYKDDDDK tag
- pre-purification of cell lysates is not necessary before screening and analysis of recombinant DYKDDDDK tagged protein expression by ELISA using the plates
- after immobilizing DYKDDDDK fusion proteins, the plates are useful for screening for sera antibodies to the fusion protein

Product specifications

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

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

Binding capacity[1]
Microplate was saturated with Recombinant Human Flag Ubiquitin at a concentration close to 6.0 µg/ml (600 ng/well) in an ELISA format using a Mab anti Ubiquitin-Biotin plus Streptavidin-HRP as detector and TMB as substrate (see technical note no. 49 for data and experiment details).
The Biomat Mouse Monoclonal anti-DYKDDDDK microplate shows a nominal **binding capacity ~ 600 ng/well of Recombinant Human Flag Ubiquitin**

Sensitivity[2]
Recombinant Human Flag Ubiquitin was detected at a concentration significantly above background in an ELISA format using a Mab anti Ubiquitin-Biotin plus Streptavidin-HRP as detector and TMB as substrate (see technical note no. 49 for data and experiment details).
The Biomat mouse monoclonal anti- DYKDDDDK microplate shows a **sensitivity of ~ 12 ng/well of Recombinant Human Flag Ubiquitin**

Uniformity
Microplates show a **CV% less than 5** when used as a sandwich of Recombinant Human Flag Ubiquitin in an ELISA format using a Mab anti Ubiquitin-Biotin plus Streptavidin-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%
Imidazole	≤ 62.5 mM
Deoxycholic Acid	**DO NOT** use any reagent that contains this detergent since it will inhibit the anti DYKDDDDK antibody from binding to DYKDDDDK tag proteins
SDS	**DO NOT** use any reagent that contains this reagent in the loading and wash buffer, since it will denature the anti-DYKDDDDK antibody and destroy its ability to bind DYKDDDDK 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 anti DYKDDDDK plate than ones with higher M.W.

Note[2]: The sensitivity is improved by increasing the incubation time between the anti DYKDDDDK antibody and the DYKDDDDK tag protein. It is possible to bring the incubation time to O/N for 12-24 h at + 4 °C.

TECHNICAL NOTES N. 49 – binding capacity and sensitivity test

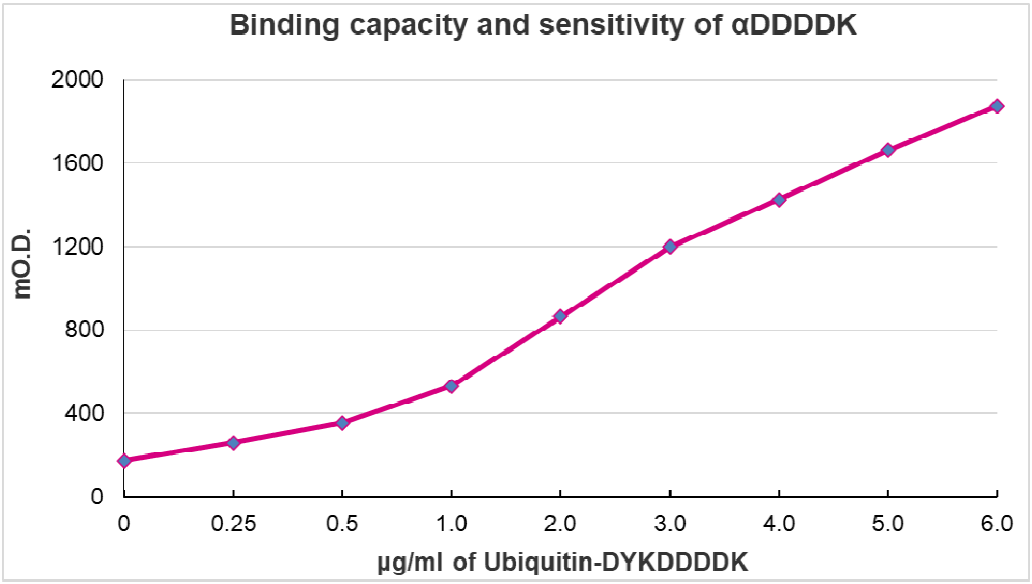
1. Prepare a standard curve of purified Recombinant Human Flag Ubiquitin (*BostonBiochem* code #U-120), from 0.25 to 6.0 µg/ml, diluted in Sample Diluent (*Biomat* code 400-1);
2. Add 100 µl of different concentrations of purified Recombinant Human Flag Ubiquitin to the wells of monoclonal mouse anti-DYKDDDDK 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



TECHNICAL NOTES N. 50 – General ELISA procedures using anti-DYKDDDDK 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 DYKDDDDK-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-DYKDDDDK tag wells and incubate for 2 h 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 DYKDDDDK-tagged proteins in samples.
Before test, the operator should do preliminary experiments to set up a standard curve of DYKDDDDK-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-DYKDDDDK tag wells and incubate for 2 h 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 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.