MAB ANTI GST (Glutathione S-Transferase) COATED SURFACE

The Biomat product is a 96 well coated microplate with mouse monoclonal anti GST and a protein to block non-specific binding sites and to maintain stable activity.
These plates are designed to specifically bind GST tagged proteins and GST. The plates can be used for isolation of GST-tagged proteins from bacterial lysates and then used for subsequent ELISA protocols.

Features of mouse monoclonal anti-GST antibody coated plates:

- ideal to bind proteins with GST (Glutathione S-Transferase) tag
- pre-purification of cell lysates is not necessary before screening and analysis of recombinant GST-tagged protein expression by ELISA using the plates
- after immobilizing GST 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-GST 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 GST at a concentration of 1.0 µg/ml (100 ng/well) in an ELISA format using goat anti GST-HRP as detector and TMB as substrate (see technical note no. 44 for data and experiment details).
The Biomat Mouse Monoclonal anti-GST microplate shows a nominal **binding capacity of ~ 100 ng /well of GST**

Sensitivity[1]
GST was detected at a concentration significantly above background in an ELISA format using goat anti GST-HRP as detector and TMB as substrate (see technical note no. 44 for data and experiment details).
The Biomat Mouse Monoclonal anti-GST microplate shows a **sensitivity of ~ 1 ng/well of GST**

Uniformity
Microplates show a **CV% less than 7** when used as a sandwich of GST in an ELISA format using goat anti GST-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-GST antibody and destroy its ability to bind GST 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.

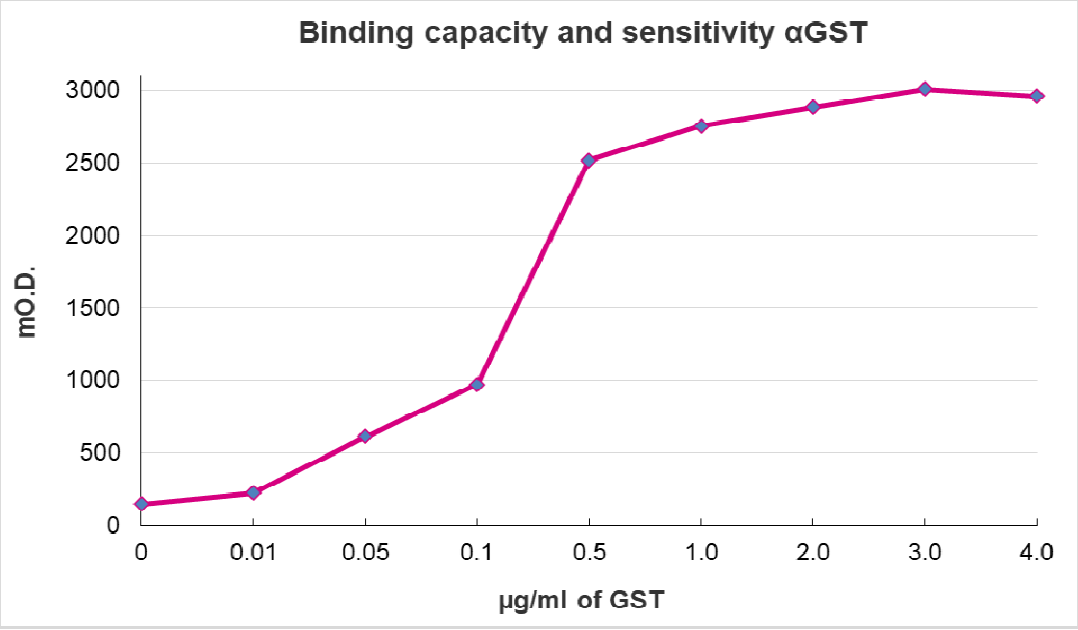
Note[1]: The binding capacity and sensitivity varies, depending on protein size and structure. Generally, proteins with low molecular weight (M.W.) can be more sensitive and more bounded to anti GST plate than ones with higher M.W.

TECHNICAL NOTES N. 44 – binding capacity and sensitivity test

1. Prepare a standard curve of purified recombinant GST (*GenScript* code Z02039-1), from 0.01 to 4.0 µg/ml, diluted in Sample Diluent (*Biomat* code 400-1*)*;
2. Add 100 µl of different concentrations of purified recombinant GST to the wells of monoclonal mouse anti-GST 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 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;
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 a GST concentration of 1.0 µg/ml.
This concentration means the well binding capacity we can express as:
- µg/well = 0.1 (100 ng/well)
- pMol/well= 3.4 (this result is calculated considering the GST M.W. = 29,000 Da)

The microplate sensitivity was calculated as the lowest GST concentration higher than the mean optical density plus 5 S.D. of 0 µg/ml GST concentration.
Our experiment gave the following results:
- 0 µg/ml GST optical density mean (coming from 8 replicates) = 0.144
- standard deviation = 0.017
- mean + 5 S.D. = 0.085
- sensitivity = 1.2 ng/well of GST



TECHNICAL NOTES N. 45 – General ELISA procedures using anti-GST 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

1) To determine the approximate concentration of the GST-tagged protein, generate a standard curve using purified recombinant GST (0 – 0.01 – 0.05 – 0.25 – 1.0 µg/ml)
2) We suggest to prepare 2 dilutions of the cell lysate containing GST-tagged protein or of an intermediate purification fraction. Take in account the sensitivity and the binding capacity of anti-GST coated plate.
3) Add 100 µl of standard GST points and diluted samples in duplicate into anti-GST wells 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 a **polyclonal** anti-GST-HRP and incubate for 60 minutes at room temperature
6) Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* four times
7) Add 100 µl/well of TMB substrate solution (*Biomat* code 500-1) and incubate 15 minutes at room temperature
8) 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
9) 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. The initial dilution has to be taken into consideration when reading the results from the graph. Also consider the molecular weight of the GST-protein being analyzed compared to the molecular weight of the GST when calculating the concentration of the GST-protein.
If the sample optical density value is higher than the upper limit of the standard curve, the sample should be re-diluted and the experiment rerun.

Procedure 2

1) To determine the approximate concentration of the GST-tagged protein, generate a standard curve using purified recombinant GST (0 – 0.01 – 0.05 – 0.25 – 1.0 µg/ml)
2) We suggest to prepare 2 dilutions of the cell lysate containing GST-tagged protein or of an intermediate purification fraction. Take in account the sensitivity and the binding capacity of anti-GST coated plate.
3) Add 100 µl of standard GST points and diluted samples in duplicate into anti-GST wells 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 an appropriate dilution of **polyclonal** anti-GST (primary antibody) and incubate for 60 minutes at room temperature
6) Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* four times
7) Add 100 µl/well of an appropriate dilution of the labelled HRP **mouse antibody** anti primary antibody and incubate for 60 minutes at room temperature
8) Empty the wells and wash with Wash Buffer (*Biomat* code 200-3*)* four times
9) Add 100 µl/well of TMB substrate solution (*Biomat* code 500-1) and incubate 15 minutes at room temperature
10) 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
11) 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. The initial dilution has to be taken into consideration when reading the results from the graph. Also consider the molecular weight of the GST-protein being analyzed compared to the molecular weight of the GST when calculating the concentration of the GST-protein.
If the sample optical density value is higher than the upper limit of the standard curve, the sample should be re-diluted and the experiment rerun.

Procedure 3

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 GST-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-GST 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.