MAB ANTI GST (Glutathione S-Transferase) COATED SURFACE

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.