MAB ANTI HIS (Polyhistidine)-tag COATED SURFACES

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.