IMMUNOTOXICITY ELISA COATED SURFACES

The Biomat products are 96 well microplates coated with KLH (*Keyhole Limpet Hemocyanin*), Tetanus Toxoid, DNP (Dinitrophenol) or TNP (Trinitrophenol) and a protein to block non-specific binding sites and to maintain stable activity. These plates are ideal for to set up ELISA IgM and IgG assays to be used as biomarkers of immunotoxicity.

KLH can be either used as vaccine carrier protein acting as the hapten carrier part of the vaccine component or as a highly immunogenic antigen in order to assess the immune competence of an organism and as a carrier of low molecular mass peptide and haptens, such as oligosaccharides, gangliosides or (glyco)peptides, designed to facilitate antibody production.
Dinitrophenol and trinitrophenol are haptens that can be attached to carrier proteins such as KLH (Keyhole Limpet Hemocyanin) or ovoalbumin; when one of this complex is injected into appropriate animal models, produce an immune response which is measured as changes in the levels of anti DNP (or anti TNP) IgM and IgG. Thanks to these changes, the researchers can assess the impact of pharmacologic or genetic manipulations on the studied immune system.
Tetanus, commonly called lockjaw, is a serious bacterial disease that affects muscles and nerves. It is characterized by muscle stiffness that usually involves the jaw and neck that then progresses to involve other parts of the body. This disease is caused by neurotoxin from deep wound infection with *Clostridium tetani.* The use of this product can find application to set up assays where the level of anti-tetanus toxoid antibodies present in biological samples are measured spectrophotometrically.
Anti-KLH, anti-tetanus toxoid, anti-DNP and anti-TNP IgM and IgG are routinely used as biomarkers of immunotoxicity.

In preclinical studies, animals are immunized with an antigen such as KLH while being dosed with a drug candidate. The levels of anti-KLH IgM and IgG are determined in serum or plasma collected at 5-7 or 14-21 days and compared with those in a control group that was not exposed to the drug. A decrease in anti-KLH levels in the treatment group provides evidence of immunosuppression.

General instructions for the use of microplates (on request Biomat can supply microplates for fluorescent and chemiluminescent assays)

1. The microplates have to be used to set up a solid phase enzyme-linked immunosorbent assay (ELISA). The assay will use microplates for solid phase immobilization and horseradish peroxidase (HRP) conjugated anti-animal IgG or IgM antibodies for detection. Test serum or plasma samples are diluted and incubated in the microplate wells for 30-60 minutes at room temperature. The microplate wells are subsequently washed, and HRP-conjugate is added and incubated for 30-60 minutes at room temperature. Immunized animal IgG or IgM are thus sandwiched between immobilized antigen (KLH, Tetanus Toxoid, DNP, TNP) and the detection conjugate. The wells are then washed to remove unbound HRP-labelled antibodies, and TMB reagent is added and incubated for 15 minutes at room temperature. This results in the development of a blue colour. Colour development is stopped by the addition of Stop Solution, changing the colour from blue to yellow, and optical density is measured spectrophotometrically at 450nm. The level of animal IgG or IgM anti antigen (KLH, Tetanus Toxoid, DNP, TNP) is proportional to the optical density (O.D.) of the test sample.

2. Sample preparation: depending on the animal to be studied, it will be useful to determine the correct serum/plasma dilution under assessment, so that the dilution results in an optically readable density value at the spectrophotometer (preferably between 1,000 and 2,0000 m O.D.). In our internal evaluation we evaluated the determination of mouse IgG anti KLH, DNP, TNP and Tetanus Toxoid through the use of specific monoclonal antibodies. Considering a concentration of 2.0 mg/ml of IgG in a normal mouse serum, we have constructed the relevant titration curves expressed as both antibody weight (µg/ml) and the corresponding serum/plasma dilution factor (see the technical notes of the respective microplates).

3. *Biomat* suggests to study a working HRP-conjugate dilution as follow:
    a) dilute the HRP-conjugate using an appropriate conjugate diluent
    b) choose the HRP-conjugate dilution that allows to get an optical density less than 0.1 O.D. for an animal non immunized and an optical density within range 1.000-2.000 m O.D. for a positive immunized animal

Materials provided upon request

Biomat can provide the following reagents to set up the ELISA assay:
- Sample Diluent
- 20x Wash Solution
- Conjugate Diluent
- TMB reagent
- Stop Solution