

KLH (Keyhole Limpet Hemocyanin) COATED SURFACE

The Biomat product is a 96 well coated microplate with KLH (Keyhole Limpet Hemocyanin) and a protein to block non-specific binding sites and to maintain stable activity.

KLH is a large, multisubunit, oxygen-carrying, metalloprotein that is found in the hemolymph of the Californian giant keyhole limpet *Megatura crenulata*. Its hemocyanin has been recognized as a potent immunoactivator and therefore is widely used in research and clinical studies.

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.

Example of applications:

- assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing
- determination of immune status relative to controls
- quantifying and standardizing vaccines batches and protocols

Product specifications

Available configurations

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

Coating

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

Binding capacity

Microplate was saturated with mouse IgG anti KLH at a concentration of 0.250 µg/ml (25 ng/well) in an ELISA format using goat anti mouse IgG-HRP as detector and TMB as substrate (see Technical notes 39 and Figure 1 and 2 for data and experiment details).

The Biomat KLH microplate shows a nominal **binding capacity of ~ 0.250 µg/ml of mouse IgG antiKLH**

Uniformity

Microplates show a **CV% less than 5** when used as a sandwich of mouse IgG anti KLH in an ELISA format using goat anti mouse IgG -HRP as detector and TMB as substrate.

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.

TECHNICAL NOTES N. 39

Binding capacity test

1. Add 100 µl of different concentrations of monoclonal mouse IgG anti KLH (*BioLegend* code 408502) at 0.5 mg/ml), from 0.0005 to 0.500 µg/ml, diluted in Sample Diluent, (*Biomat* code 400-1-100) to the wells of KLH coated plate and incubate for 60 minutes at room temperature
2. Empty the wells and wash with Wash Buffer, (*Biomat* code 200-1-100) four times
3. Add 100 µl/well of goat anti-mouse IgG -HRP (*Jackson ImmunoResearch* code 115-035-003), diluted 1:25,000 in Diluent for HRP conjugate, (*Biomat* code 400-2-100) and incubate for 60 minutes at room temperature
4. Empty the wells and wash with Wash Buffer, (*Biomat* code 200-1-100) four times
5. Add 100 µl/well of TMB substrate solution, (*Biomat* code 500-1-100) and incubate 15 minutes at room temperature

6. Stop the substrate reaction by adding 100 μ l /well of sulphuric acid, (*Biomat* code 600-1-100) and read the optical density values at 450 nm

The data show that a plateau has got starting with a mouse IgG anti KLH concentration of 0.250 μ g/ml.

This concentration means the well binding capacity we can express as:

- μ g/well = 0.025 (25 ng/well)

Figure 1

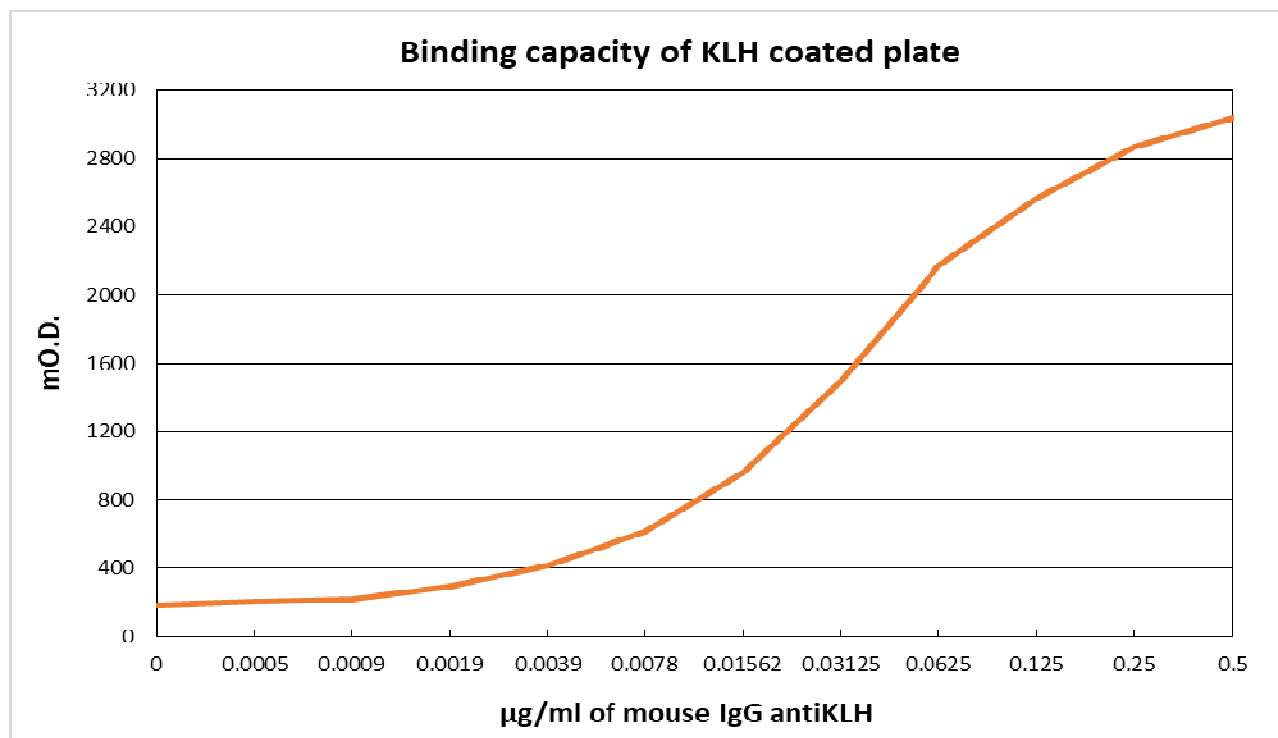


Figure 2: the figure gives an idea of the dilution factor to apply to the serum/plasma of the immunized mouse under evaluation; where k means a dilution of 1:1,000

