**Mouse Anti-KLH IgM ELISA**

**LIFE DIAGNOSTICS, INC., CATALOG NUMBER: KLHM-1**

**INTRODUCTION**

Measurement of KLH (keyhole limpet hemocyanin) induced anti-KLH antibody levels allows quantitative evaluation of the immune response (ref 1). This ELISA is designed for the rapid and quantitative measurement of mouse anti-KLH IgM levels in mouse serum or plasma. A companion ELISA, catalog number KLHG-1, can be used for measurement of mouse anti-KLH IgG.

**PRINCIPLE OF THE ASSAY**

The mouse anti-KLH IgM test kit is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses KLH for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-mouse IgM antibodies for detection. Test serum or plasma samples are diluted and incubated in the microtiter wells for 1 hour. The microtiter wells are subsequently washed, and HRP conjugate is added and incubated for 45 minutes. Anti-KLH IgM molecules are thus sandwiched between immobilized KLH and the detection antibody conjugate. The wells are then washed to remove unbound HRP-labeled antibodies, and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow. Optical density is measured spectrophotometrically at 450 nm. The concentration of anti-KLH IgM is proportional to the optical density of the test sample.

**MATERIALS AND COMPONENTS**

**Materials provided with the kit:**
- KLH Coated 96-well Plate (provided as 12 strips of 8 wells)
- Enzyme Conjugate Reagent, 11 ml
- Reference Standard1 (lyophilized)
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD60-1, 60 ml
- TMB Reagent (One-Step): TMB11-1, 11 ml
- Stop Solution (1N HCl): SS11-1, 11 ml

**Materials required but not provided:**
- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-plate incubator/shaker mixing speed of 150 rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450 nm
- Graph paper (PC graphing software is optional)

**STORAGE**

- The reference standard should be stored at -20°C for optimal stability.
- All remaining kit components should be stored at 4°C

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1 Mouse anti-KLH IgM levels are measured in nominal units and are calibrated with reference anti-KLH mouse serum at Life Diagnostics, Inc.
ASSAY PROCEDURE
1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 1 hour.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 µl/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
6. Add 100 µl of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 µl of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS
1. Calculate the average absorbance values (A\textsubscript{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of anti-KLH IgM in u/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of anti-KLH IgM in the serum/plasma sample.
5. PC graphing software may be used for the above steps.
6. If the OD\textsubscript{450} values of samples fall outside the standard curve when tested at a dilution of 500, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE
A typical standard curve with optical density readings at 450nm on the Y-axis against anti-KLH IgM concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.

<table>
<thead>
<tr>
<th>Anti-KLH IgM (u/ml)</th>
<th>A\textsubscript{450}</th>
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<tbody>
<tr>
<td>100</td>
<td>3.145</td>
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<tr>
<td>50</td>
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<tr>
<td>25</td>
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<tr>
<td>12.5</td>
<td>0.581</td>
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<tr>
<td>6.25</td>
<td>0.330</td>
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