Human Anti-KLH IgM ELISA
Life Diagnostics, Inc., Catalog Number: KLHM-20

USE STATEMENT

This kit is for research use only. Under no circumstances may it be used for diagnostic purposes.

INTRODUCTION

Keyhole limpet hemocyanin (KLH) is a potent immunostimulant used in research studies and clinical applications. Determination of anti-KLH antibody levels allows assessment of immune system regulation. This kit allows quantitative measurement of anti-KLH IgM levels in serum, plasma, and other fluids.

PRINCIPLE OF THE ASSAY

The assay uses KLH as capture reagent coated on microtiter wells, and horseradish peroxidase (HRP) conjugated anti-human IgM for detection. Standards and diluted samples are incubated in microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. Anti-KLH IgM molecules are thus sandwiched between immobilized KLH and the HRP conjugate. The wells are then washed to remove unbound HRP-labeled antibodies. TMB 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. Absorbance is measured at 450 nm. The concentration of anti-KLH IgM is proportional to the absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- KLH Coated 96-well plate (12 x 8 well strips)
- Anti-Human IgM HRP Conjugate, 11 ml
- Anti-KLH IgM Stock[A] (lyophilized)
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD50-1, 50 ml
- TMB Reagent: 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
- Plate incubator/shaker with mixing speed of 150 rpm
- Plate washer
- Plate reader with an absorbance range of 0-4 at 450 nm
- Graphing software

[A] The reference standard provided with the kit was calibrated using affinity purified human anti-KLH IgM prepared at Life Diagnostics, Inc. IgM content was measured using a human IgM SPARCL assay developed at Life Diagnostics Inc.

STORAGE

The anti-KLH IgM stock should be stored at -20°C or lower. The remainder of the kit should be stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. The kit will remain stable for six months from the date of purchase provided that the components are stored as described.

GENERAL INSTRUCTIONS

1. Please read the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (25°C) before use.
3. The optimal sample dilution should be determined empirically.
4. Optimal results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. Standards should be used within 30 min of preparation.
2. The anti-KLH IgM stock is provided in lyophilized form. Reconstitute as directed on the vial label ***(the reconstituted stock should be frozen at -20°C if additional use is intended)***.
3. Label 6 polypropylene or glass tubes as 1000, 500, 250, 125, 62.5 and 31.25 ng/ml.
4. Into the tube labeled 1000 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of anti-KLH IgM and mix gently. This provides the 1000 ng/ml standard.
5. Dispense 250 µl of diluent into the tubes labeled 500, 250, 125, 62.5 and 31.25 ng/ml.
6. Prepare a 500 ng/ml standard by diluting and mixing 250 µl of the 1000 ng/ml standard with 250 µl of diluent in the tube labeled 500 ng/ml.
7. Similarly prepare the remaining standards by serial dilution.

SAMPLE PREPARATION

The optimal sample dilution should be determined empirically. Studies at Life Diagnostics, Inc., using ascites fluid samples, suggest that a 500-fold dilution is a reasonable starting point. Use the YD50-1 buffer provided with the kit for dilution.

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.
3. Incubate on a plate shaker at 150 rpm/25°C for 45-minutes.
4. Aspirate the contents of the microtiter wells and wash the wells five times with 1x wash solution using a plate washer (400 µl/well).
5. Strike the wells sharply onto absorbent paper to remove all residual wash solution.
6. Add 100 µl of diluted HRP conjugate into each well.
7. Incubate on a plate shaker at 150 rpm/25°C for 45-minutes.

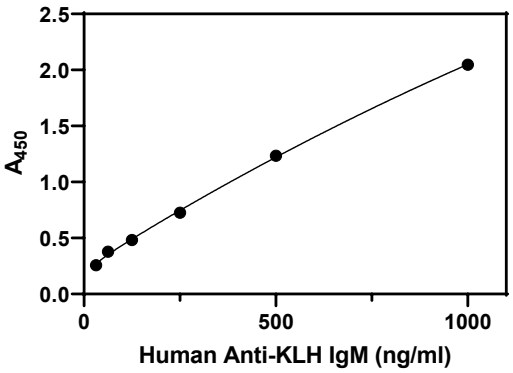
8. Wash as detailed above.
9. Dispense 100 µl of TMB into each well.
10. Incubate on a plate shaker at 150 rpm/25°C for 20-minutes.
11. Stop the reaction by adding 100 µl of stop solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Measure absorbance at 450 nm with a microtiter plate reader within five minutes.

CALCULATION OF RESULTS

1. Using graphing software construct a standard curve by plotting the absorbance of the standards versus concentration.
2. Fit standard data to a two-site, total and non-specific binding model (others may be used at the discretion of the researcher) and derive the concentration of anti-KLH IgM in the samples.
3. Multiply the derived concentration by the dilution factor(s) to determine the actual concentration of anti-KLH IgM in the original sample.
4. If absorbance values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

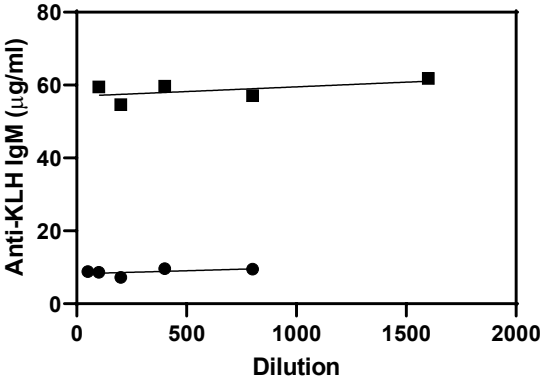
A typical standard curve is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.



Anti-KLH IgM (ng/ml)	A_{450}
1000	2.048
500	1.235
250	0.726
125	0.482
62.5	0.378
31.25	0.258

ASSAY PERFORMANCE

Parallelism: To assess performance of the assay, two samples containing anti-KLH IgM at concentrations of 58.5 and 8.7 µg/ml were serially diluted from 50- to 1600-fold to produce values within the dynamic range of the assay.



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For technical assistance please email us at techsupport@lifediagnostics.com