Version 23-04/25

User's Manual

KLH IgM (Monkey) ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This test kit allows rapid and quantitative measurement of anti-KLH IgM levels in serum or plasma.

General Description

Drug candidates are routinely screened for evidence of immune system regulation during the discovery process. It is important that the immune response is not enhanced or diminished since such effects may lead to hypersensitivity or increased susceptibility to infection. Determination of a drug candidate's effects on anti-KLH (keyhole limpet hemocyanin) antibody levels allows easy assessment of immune system regulation. Animals are immunized with KLH while undergoing drug treatment, and serum is collected at appropriate times post immunization. Typically, serum collected 5-7 days after immunization is used for measurement of anti-KLH IgM levels, and serum collected 14+ days post immunization is used to measure anti-KLH IgG levels. Comparison of anti-KLH IgG or IgM levels in drug treated versus control groups reveals effects of the drug on the immune response.

Principles of Testing

The monkey anti-KLH IgM test kit is a solid phase enzyme-linked immunosorbent assay (ELISA). It uses KLH for solid phase (microtiter wells) immobilization and a horseradish peroxidase (HRP) conjugated goat anti-monkey IgM antibody for detection. Serum or plasma samples are diluted and incubated in the microtiter wells for 45 minutes. 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, and optical density is measured spectrophotometrically at 450 nm. The concentration of anti-KLH IgM is proportional to the optical density.

Reagents And Materials Provided

1. KLH Coated 96-well Plate (provided as 12 strips of 8 wells)
2. Anti Monkey IgM HRP Conjugate, 11 mL
3. Anti-KLH IgM Stock (lyophilized)
4. 20x Wash Solution, 50 mL
5. Diluent, 50 mL
6. TMB Reagent (One-Step), 11 mL
7. Stop Solution (1N HCl), 11 Ml

Materials Required But Not Supplied

1. Precision pipettes and tips
2. Distilled or deionized water
3. Polypropylene or glass tubes
4. Vortex mixer
5. Absorbent paper or paper towels
6. Micro-plate incubator/shaker mixing speed of ~150 rpm
7. Plate washer
8. Plate reader with an optical density range of 0-4 at 450nm
9. Graph paper (PC graphing software is optional)

Storage

On receipt, the anti-KLH IgM standard stock should be stored frozen at -20°C or lower. The remainder of the kit should be stored at 2-8°C, and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. DO NOT FREEZE THE HRP CONJUGATE OR TMB SOLUTIONS. Test kits will remain stable for six months from the date of purchase provided that the components are stored as described.

Specimen Collection And Preparation

The optimal sample dilution should be determined empirically. However, studies at Creative Diagnostics, Inc. suggest that a 500-fold dilution is a reasonable starting point. In order to achieve high dilutions we suggest that a serial dilution strategy be used. If, for example, a 500-fold sample dilution is desired the following procedure should used. This approach minimizes diluent usage and favors accurate and precise sample dilution.

1. Dispense 48 µL and 237.5 µL of diluent into separate tubes.
2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 48 µL of diluent. This provides a 25 fold diluted

sample.

3. Mix 12.5 µL of the 25 fold diluted sample with the 237.5 µL of diluent in the second tube. This provides a 500 fold dilution of

the sample.

4. Repeat this procedure for each sample to be tested.

Do not use dilutions less than 100-fold (i.e., do not use dilutions of 50-fold).

Reagent Preparation

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. Working 400–12.5 ng/mL anti-KLH IgM standards should be used within 1 hour of preparation.
2. The anti-KLH IgM stock is provided in lyophilized form. Reconstitute as directed on the vial label (the reconstituted standard should be aliquoted and frozen at -20°C after reconstitution if additional use is intended).
3. Label 6 polypropylene or glass tubes as 400, 200, 100, 50, 25 and 12.5 ng/mL.
4. Into the tube labeled 400 ng/mL, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of anti-KLH IgM stock (also detailed on the vial label) and mix gently. This provides the 400 ng/mL standard.
5. Dispense 250 µL of diluent into the tubes labeled 200, 100, 50, 25 and 12.5 ng/mL.
6. Prepare a 200 ng/mL standard by diluting and mixing 250 µL of the 400 ng/mL standard with 250 µL of diluent in the tube labeled 200 ng/mL.
7. Similarly prepare the 100, 50, 25 and 12.5 ng/mL standards by serial 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 (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
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 HRP conjugate into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°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 (18-25°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

1. Using graphing software, construct a standard curve by plotting the absorbance values for the standards versus the log10 of the anti-KLH IgM concentration in ng/mL.
2. Fit the data using a variable slope, four-parameter logistic curve.
3. Derive the corresponding concentration of anti-KLH IgM in the samples from the standard curve (derive the concentration from the antilog).
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of anti-KLH IgM in the serum/plasma sample.

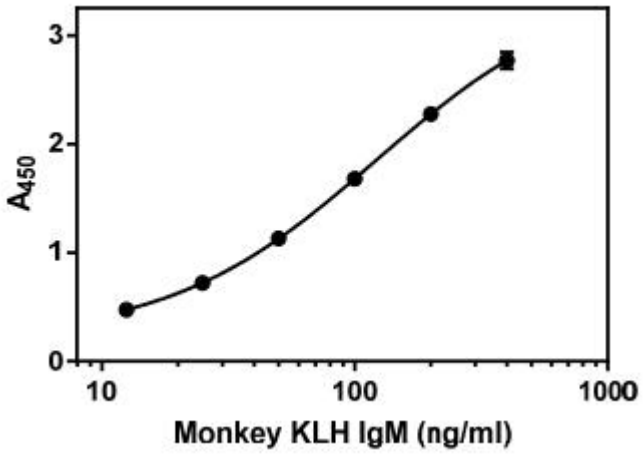
5. If the OD450 values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

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. Each user should obtain his or her data and standard curve in each experiment.

Anti-KLH IgM (ng/ml)	A_{450}
400	2.770
200	2.278
100	1.681
50	1.130
25	0.722
12.5	0.474



Precautions

1. Please read the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (18-25°C) before use.
3. The optimal sample dilution should be determined empirically. Do not use dilutions less than 100-fold (i.e., do not use dilutions of 50-fold).
4. Optimal results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

Limitations

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding

of and in accordance with the instructions detailed above.

2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.