KAMIYA BIOMEDICAL COMPANY

Mouse Anti-Keyhole Limpet Hemocyanin (KLH) IgG ELISA

For the quantitative determination of anti-KLH IgG in mouse serum and plasma

Cat. No. KT-566

For research use only.

PRODUCT INFORMATION

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PRODUCT

The **K-*ASSAY*®** Mouse Anti-KLH IgG ELISA is for the quantitative determination of Anti-KLH IgG in mouse serum and plasma. For research use only.

INTRODUCTION

Measurement of KLH induced anti-KLH antibody levels allows quantitative evaluation of the immune response. This ELISA is designed for the rapid and quantitative measurement of mouse anti-KLH IgG levels in serum or plasma.

PRINCIPLE

The mouse anti-KLH IgG ELISA 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 IgG 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 IgG 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 IgG is proportional to the optical density of the test sample.

COMPONENTS

- KLH coated 96-well plate (provided as 12 strips of 8 wells)
- Enzyme Conjugate Reagent, 11 mL
- Reference calibrator (lyophilized)
- 20X Wash Solution, 50 mL
- Diluent, 60 mL
- TMB Reagent (One-Step), 11 mL
- Stop Solution (1N HCl), 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes and tips
- Distilled or de-ionized 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 calibrator should be stored at -20℃ for optimal stability. All remaining components should be stored at 4℃. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kit will remain stable until the expiration date provided that the components are stored as described above.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. This kit is designed to measure anti-KLH IgG levels in serum or plasma collected 14 days after immunization with KLH.
3. All reagents should be allowed to reach room temperature (25℃) before use.

4. The optimal sample dilution should be determined empirically. However, studies suggest an initial sample dilution of 20,000-fold works well for most 14-day post immunization samples. Please do not use dilutions less than 25-fold.
5. Optimum results are achieved if, at each step, reagents are pipetted into 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 de-ionized water.

CALIBRATOR PREPARATION

1. The mouse anti-KLH IgG calibrator is provided as a lyophilized stock. Reconstitute with 0.1 mL of distilled or de-ionized water. The reconstituted calibrator is stable at 4°C for one week but should be aliquoted and frozen at -20°C after reconstitution if future use is intended.
2. Label 5 polypropylene or glass tubes as 100, 50, 25, 12.5 and 6.25 u/mL
3. In the tube labeled 100 u/mL prepare the 100 u/mL calibrator by adding 488.6 µL of diluent to 11.4 µL of reconstituted calibrator and mix gently.
4. Dispense 250 µL of diluent into the remaining tubes.
5. Prepare a 50 u/mL calibrator by diluting and mixing 250 µL of the 100 u/mL calibrator with 250 µL of diluent in the tube labeled 50 u/mL.
6. Similarly prepare the 25, 12.5 and 6.25 u/mL calibrators by serial dilution.

SAMPLE PREPARATION

General Note: Studies indicate that anti-KLH IgG is present in serum from KLH immunized mice at concentrations of ~750,000 u/mL. In order to obtain values within range of the calibration curve, we suggest samples initially be diluted 20,000 fold using the following procedure for each sample tested:

1. Dispense 248 µL and 318 µL of diluent into separate tubes.
2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 248 µL of diluent. This provides a 125 fold diluted sample.
3. Mix 2 µL of the 125 fold diluted sample with 318 µL of diluent in the second tube. This provides a 20,000-fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators 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 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

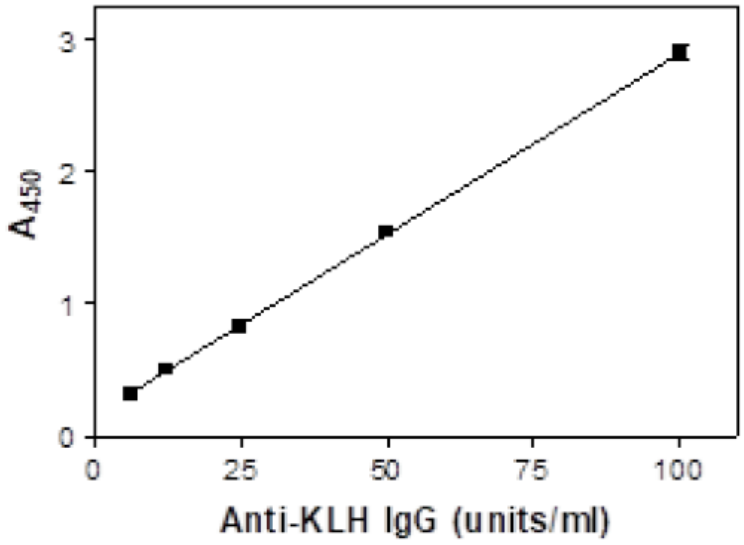
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical 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 IgG in u/mL from the calibration curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration for anti-KLH IgG in the serum/plasma sample.
5. PC graphing software may be used for the above steps.

6. If the OD_{450} values of samples fall outside the calibration curve when tested at a dilution of 20,000, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density readings at 450 nm on the Y-axis against anti-KLH IgG 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.

Anti-KLH IgG (u/mL)	A_{450}
100	2.903
50	1.542
25	0.821
12.5	0.509
6.25	0.312



LIMITATIONS OF THE PROCEDURE

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

FOR RESEARCH USE ONLY

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