KAMIYA BIOMEDICAL COMPANY

Mouse Anti-Keyhole Limpet Hemocyanin (KLH) IgG ELISA

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

Cat. No. KT-566

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

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PRODUCT

The ***K-ASSAY®*** Mouse KLH IgG ELISA is for the quantitative determination of KLH IgG in mouse 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

- Microtiter Plate: KLH coated 96-well plate (12 strips of 8 wells)
- Enzyme Conjugate Solution: 11 mL
- Calibrator: Lyoph.
- Diluent Buffer: 60 mL
- TMB Solution: 11 mL
- Stop Solution: 11 mL, 1N HCl
- Wash Buffer (20x): 50 mL

Materials or Equipment required but not provided

- Plate reader (450 nm)
- Micropipette and tips
- De-ionized water
- Graph paper (PC software is optional)
- Paper towels
- Polypropylene or glass tubes
- Vortex mixer
- Plate shaker/incubator
- Plate washer

STORAGE

Store at 4℃. Calibrators should be stored at -20℃ for optimal stability. Microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. The kit is stable until the expiration date when stored as noted in this section.

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 mouse serum or plasma collected 14 days after immunization with KLH.
3. All reagents should be allowed to reach room temperature (18-25℃) before use.
4. The optimal sample dilution should be determined empirically. However, studies suggest an initial sample dilution of 20,000 fold. 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.

PREPARATION OF REAGENTS

Wash Buffer
The wash solution is provided as 20x stock. Prior to use dilute the contents of the bottle (50 mL) with 950 mL of distilled of deionized water.

Calibrator
1. The mouse anti-KLH IgG calibrator is provided as lyophilized stock. Reconstitute with 100 µL of distilled or deionized water. The reconstituted calibrator is stable at 4°C for one week but should be aliquoted and stored 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 units/mL (u/mL).
3. Into the tube labelled 100 u/mL, pipette the volume of diluent detailed on the anti-KLH IgG calibration vial label. Then add the indicated volume of anti-KLH IgG calibrator (shown on the anti-KLH IgG calibrator vial label) and mix gently. This provides the 100 u/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labelled 50, 25, 12.5, and 6.25 u/mL.
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 labelled 50 u/mL.
6. Similarly prepare the 25, 12.5, and 6.25 u/mL calibrators by serial dilution.

SAMPLE PREPARATION
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 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.

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 (18-25°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 (18-25°C) for 45 minutes.
8. Wash as detailed in 4 and 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 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 for each set of calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each 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 values of samples fall outside the calibration curve when tested at a dilution of 500, samples should be diluted appropriately and re-tested.

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

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com