KAMIYA BIOMEDICAL COMPANY

Rat Anti-Keyhole Limpet Hemocyanin (KLH) IgG ELISA with Controls

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

Cat. No. KT-570

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

PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** Rat KLH IgG ELISA is for the quantitative determination of KLH IgG in rat serum or plasma.

PRINCIPLE
The Rat 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-rat IgG antibodies for detection. Test 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 30 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: 30 mL
- TMB Solution: 11 mL
- Stop Solution: 11 mL, 1N HCl
- Wash Buffer (20x): 50 mL
- Rat KLH IgG control: Lyoph.

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℃. The calibrator and control should be stored at -20℃. 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 Rat serum or plasma collected 14 days after immunization with KLH. At this point the immune response originates almost exclusively from IgG.
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 100 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.

Diluent
The diluent is provided as 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one volume of the 10x stock with nine volumes of distilled or deionized water.

Control
Reconstitute the lyophilized rat anti-KLH IgG control with the volume of distilled or deionized water indicated on the vial label. The concentration range of rat anti-KLH IgG after reconstitution is shown on the vial label. The assay value of the control should be within the specified range. Discard any remaining control after use.

Calibrator
1. The Rat anti-KLH IgG calibrator is provided as lyophilized stock. Reconstitute with volume of diluent indicated on the vial label to give a 500 ng/mL solution of rat anti-KLH IgG. The reconstituted calibrator is stable at 4℃ for one week but should be aliquoted and stored frozen at -20℃ after reconstitution if future use is intended.
2. Label 6 polypropylene or glass tubes as 250, 125, 62.5, 31.2, and 15.6, and pipette 250 µL of diluent into each tube.
3. Into the tube labeled 250 ng/mL, pipette and mix 250 µL of the reconsituted anti-KLH IgG calibrator. This provides the 250 ng/mL calibrator.
4. Prepare a 125 ng/mL calibrator by diluting and mixing 250 µL of the 250 ng/mL calibrator with 250 µL of diluent in the tube labelled 125 ng/mL.
5. Similarly prepare the 62.5, 31.25, 15.6, and 7.8 ng/mL calibrators by serial dilution.

SAMPLE PREPARATION
Note: Studies indicate that anti-KLH IgG is present in rat serum or plasma at concentrations up to approximately 20 µg/mL 14 days after i.v. immunization with KLH. Levels are likely higher after 14 days. In order to obtain values within range of the calibration curve, we suggest samples initially be diluted 100 fold using the following procedure for each sample tested. Optimal dilutions may need to be determined empirically.
1. Dispense 297 µL of diluent into a polypropylene or glass tube.
2. Pipette and mix 3 µL of the serum/plasma sample into the tube containing 297 µL of diluent. This provides a 100 fold diluted sample.
3. 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, controls, 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℃) 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 enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25℃) for 30 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, controls, 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 ng/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 100, 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

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