



**KAMIYA BIOMEDICAL COMPANY**

# Monkey Anti-Keyhole Limpet Hemocyanin (KLH) IgM ELISA

**For the quantitative determination of KLH-IgM in monkey serum and plasma**

**Cat. No. KT-579**

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

**PRODUCT INFORMATION****Monkey Anti-Keyhole Limpet Hemocyanin (KLH) IgM ELISA**  
**Cat. No. KT-579****PRODUCT**

The **K-ASSAY®** Monkey KLH IgM ELISA is for the quantitative determination of KLH IgM in monkey serum or plasma.

**PRINCIPLE**

The Monkey Anti-KLH IgM 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 goat anti-monkey IgM 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 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 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: 50 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°C. Calibrators should be stored at -20°C. Microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Do not freeze HRP conjugate or TMB solutions. 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. Optimal sample dilutions must be determined empirically. Do not use dilutions of less than 100-fold (i.e., do not use dilutions of 50-fold).
3. All reagents should be allowed to reach room temperature (18-25°C) before use.
4. 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 or 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.

### Calibrator

1. Working 400-12.5 ng/mL anti-KLH IgM calibrators should be used within 1 hour of preparation.
2. The Monkey anti-KLH IgM calibrator is provided as lyophilized stock. Reconstitute as directed on vial label. The reconstituted calibrator should be frozen and stored at -20°C after reconstitution if future 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 anti-KLH IgM calibration vial label. Then add the indicated volume of anti-KLH IgM calibrator (shown on the anti-KLH IgM calibrator vial label) and mix gently. This provides the 400 ng/mL calibrator.
5. Dispense 200  $\mu$ L of diluent into the tubes labelled 200, 100, 50, 25, and 12.5 ng/mL.
6. Prepare a 200 ng/mL calibrator by diluting and mixing 250  $\mu$ L of the 400 ng/mL calibrator with 250  $\mu$ L of diluent in the tube labelled 200 ng/mL.
7. Similarly prepare the 100, 50, 25, and 12.5 ng/mL calibrators by serial dilution.

## SAMPLE PREPARATION

Note: The optimal sample dilution should be determined empirically. However, studies indicate 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 be used. This approach minimizes diluent usage and favors accurate and precise sample dilution.

1. Dispense 48  $\mu$ L and 237.5  $\mu$ L of diluent into separate tubes.
2. Pipette and mix 2  $\mu$ L of the serum/plasma sample into the tube containing 48  $\mu$ L of diluent. This provides a 25 fold diluted sample.
3. Mix 12.5  $\mu$ L of the diluted sample with 237.5  $\mu$ L 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.
5. Do not use dilutions lower than 100-fold.

## PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100  $\mu$ L 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 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  $\mu$ L/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  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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 IgM in ng/mL from the calibration curve.

4. Multiply the derived concentrations by the dilution factor to determine the actual concentration for anti-KLH IgM 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 samples should be diluted appropriately and re-tested.

**FOR RESEARCH USE ONLY**

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