



KAMIYA BIOMEDICAL COMPANY

Monkey Anti-Tetanus Toxoid IgM ELISA Kit

**For the quantitative determination of Anti-Tetanus Toxoid IgM in monkey serum
or plasma**

Cat. No. KT-581

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

PRODUCT INFORMATION

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PRODUCT

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

INTRODUCTION

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 decreased since such effects may lead to hypersensitivity or increased susceptibility to infection. Determination of a drug candidate's effects on anti-tetanus toxoid antibody levels allows easy assessment of immune system regulation. Animals are immunized with tetanus toxoid while undergoing drug treatment and serum is collected at appropriate times post immunization. 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 on the immune response.

This ELISA allows rapid and quantitative measurement of anti-tetanus toxoid IgM levels in Rhesus or Cynomolgus monkey serum or plasma.

PRINCIPLE

The monkey anti-tetanus toxoid IgG ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses tetanus toxoid for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-monkey IgM antibody for detection. Serum or plasma samples are diluted and incubated in the microtiter wells for 60 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. Anti-tetanus toxoid IgM molecules are thus sandwiched between immobilized tetanus toxoid 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-tetanus toxoid IgM is proportional to the optical density of the test sample.

COMPONENTS

- Tetanus toxoid coated 96-well plate (12 strips of 8 wells)
- Enzyme Conjugate Reagent, 11 mL
- Reference calibrator (lyophilized). **Store ≤ -20°C**
- 20X Wash Solution, 50 mL
- Diluent (30 mL)
- TMB Reagent (One-Step) 11 mL
- Stop Solution (1N HCl), 11 mL

Materials or Equipment 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 or below -20 °C. All other kit components should be stored at 4 °C and the microtiter plate should be kept in a sealed bag with the desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase provided that the components are stored as described.

General Instructions

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (18-25 °C) before use.
3. The assay was designed for use with serum or plasma obtained from monkeys 5 days after immunization with tetanus toxoid, at which point the immune response originates predominantly from IgM.
4. The optimal sample dilution should be determined empirically. However, studies indicate that an initial sample dilution of 1,000 is a good starting point. **Do not test samples at dilutions below 400 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 Solution

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 de-ionized water.

Calibrator

1. The monkey anti-tetanus toxoid IgM reference calibrator is provided as lyophilized stock. Reconstitute with 200 μ L distilled or deionized water. ***(The reconstituted calibrator is stable for 1 day at 4 °C, but should be aliquoted and stored frozen at or below -20 °C after reconstitution if additional use is intended)***.
2. Label 5 polypropylene or glass tubes as 1,000, 500, 250, 125, and 62.5 ng/mL.
3. Into the tube labelled 1,000 ng/mL, pipette the volume of diluent detailed on the calibrator stock vial label. Then add the indicated volume of stock calibrator and mix gently. This provides the 1,000 ng/mL calibrator.
4. Prepare the 500 ng/mL calibrator by diluting and mixing 250 μ L of the 1,000 ng/mL calibrator with 250 μ L of diluent in the tube labelled 500 ng/mL.
5. Similarly prepare the 250, 125, and 62.5 ng/mL calibrators by serial dilution.

SAMPLE PREPARATION

General Note: The level of anti-tetanus toxoid IgM will depend on dose, route of immunization and time of sample collection. We found that anti-tetanus toxoid IgM is present in monkey serum at concentrations of 75 μ g/mL or greater. We suggest that samples initially be diluted 1,000 fold using the following procedure for each sample to be tested. Optimum dilutions must be determined empirically. Dilutions of 400 fold or lower should not be used.

1. Dilute 5 μ L of serum with 95 μ L of diluent to give a 20-fold dilution.
2. Dilute 10 μ L of the 20-fold diluted sample with 490 μ L of diluent to give a 1,000-fold dilution.

PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μ L of calibrators (2.5-0.078 ng/mL) and diluted samples into the wells (calibrators and samples should be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25 °C) for 60 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 μ 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 μ 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 to 5 above.
9. Dispense 100 μ 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 μ 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-tetanus toxoid IgM in ng/mL from the calibration curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of anti-tetanus toxoid 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 when tested at a dilution of 200, samples should be diluted appropriately and re-tested.

FOR RESEARCH USE ONLY

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