DATA SHEET

Monkey Anti-Tetanus Toxoid IgG1 ELISA Kit

For the quantitative determination of Anti-Tetanus Toxoid IgG1 in monkey serum or plasma

Cat. No. KT-582

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

PRODUCT

The K-ASSAY Monkey Anti-Tetanus Toxoid IgG1 ELISA is for the quantitative determination of Anti-Tetanus Toxoid IgG1 in monkey serum or plasma.

INTRODUCTION

Drug candidates are routinely screened for evidence of immune system regulation during the discovery process. It isimportant 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 antiKLH 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 IgG1 levels in serum or plasma. IgG1 is the major IgG subclass in monkeys.

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 mouse monoclonal anti-monkey IgG1 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 IgG1 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 IgG1 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), 2 vials. Store ≤ -20°C

• 20X Wash Solution, 50 mL

• Diluent (50 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 14 days or more after immunization with tetanus toxoid, at which point the immune response originates predominantly from IgG.

4. The optimal sample dilution should be determined empirically. Do not test samples at dilutions below 50 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 IgG1 reference calibrator is provided as lyophilized stock. Reconstitute with the volume of diluent indicated on the vial label.

2. Prepare a 2.5 µg/mL working calibrator of monkey anti-tetanus toxoid IgG1 as further directed on the reference calibrator vial label (the reconstituted calibrator should be frozen at or below -20°C after reconstitution if additional use is intended).

3. Label 5 polypropylene or glass tubes as 1.25, 0.625, 0.313 and 0.078 µg/mL and pipette 250 µL of diluent into each tube.

4. Into the tube labeled 1.25 µg/mL, pipette and mix 250 µL of the 2.5 µg/mL anti-tetanus toxoid IgG with 250 µL of diluent. This provides the 1.25 µg/mL calibrator.

5. Similarly prepare the 0.625, 0.313 and 0.156 and 0.078 µg/mL calibrators by serial dilution.

SAMPLE PREPARATION

General Note: The level of anti-tetanus toxoid IgG1 will depend on dose, route of immunization and time of sample collection. We found that anti-tetanus toxoid IgG1 is present in monkey serum at concentrations of 50 µg/mL or greater. We suggest that samples initially be diluted 200 fold using the following procedure for each sample to be tested. Optimum dilutions must be determined empirically. Dilutions of 50 fold or lower should not be used.

1. Dispense 298.5 µL of diluent into separate polypropylene or glass tubes.

2. Pipette and mix 1.5 µL of the serum/plasma sample into the tube containing 298.5 µL of diluent. This provides a 200 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 (2.5-0.078 µg/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 5-6 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 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 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 µg/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 IgG1 in µg/mL from the calibration curve.

4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of anti-tetanus toxoid IgG1 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.