KAMIYA BIOMEDICAL COMPANY

Rat Anti-Tetanus Toxoid (TT) IgG ELISA

For the quantitative determination of TT IgG in rat serum and plasma

Cat. No. KT-578

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

PRODUCT INFORMATION

Rat Anti-Tetanus Toxoid (TT) IgG ELISA
Cat. No. KT-578

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

PRINCIPLE
The Rat Anti-TT 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-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 45 minutes. Anti-TT IgG molecules are thus sandwiched between immobilized TT antigens 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-TT IgG is proportional to the optical density of the test sample.

COMPONENTS
- Microtiter Plate: TT coated 96-well plate (12 strips of 8 wells)
- Enzyme Conjugate Solution: 11 mL
- Calibrator: Lyoph. x 2 **Store ≤ -20℃**
- Diluent Buffer: 30 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℃. The calibrator stock should be stored at or below -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-TT IgG levels in rat serum or plasma collected 14 days after immunization with TT at which point the immune response originates predominately 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 2,000 fold. It is recommended that samples not be tested at dilutions below 200 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 rat anti-tetanus toxoid IgG calibrator is provided as a lyophilized stock. Reconstitute one vial with distilled or deionized water as described on the vial label to give the 100 u/mL stock. (The reconstituted calibrator should be frozen at or below -20℃ if future use is intended.)
2. Label 5 polypropylene or glass tubes as 50, 25, 12.5, 6.25, and 3.125 u/mL and pipette 250 µL of diluent into each tube.
3. Into the tube labelled 50 u/mL, pipette and mix 250 µL of the reconstituted 100 u/mL anti-tetanus toxoid IgG. This provides the 50 u/mL calibrator.
4. Similarly prepare the 25, 12.5, 6.25, and 3.125 u/mL calibrators by serial dilution.

SAMPLE PREPARATION
Note: Studies indicate that anti-TT IgG is present in rat serum or plasma at concentrations of 50,000 u/mL or greater. In order to obtain values within range of the calibration curve, we suggest samples initially be diluted 2,000 fold using the following procedure for each sample tested. Optimal dilutions may need to be determined empirically.
1. For each test sample dispense 98 µL and 243.75 µL diluent into separate tubes.
2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 98 µL of diluent. This provides a 50 fold diluted sample.
3. Pipette and mix 6.25 µL of the serum/plasma sample into the tube containing 243.75 µL of diluent. This provides a 2,000 fold diluted 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℃) 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 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 u/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-TT IgG in u/mL from the calibration curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration for anti-TT 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

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