



**KAMIYA BIOMEDICAL COMPANY**

# Rat Anti-Tetanus Toxoid (TT) IgM ELISA

**For the quantitative determination of TT IgM in rat serum and plasma**

**Cat. No. KT-577**

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

**PRODUCT INFORMATION****Rat Anti-Tetanus Toxoid (TT) IgM ELISA**  
**Cat. No. KT-577****PRODUCT**

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

**PRINCIPLE**

The Rat Anti-TT IgM 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 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-TT IgM 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 IgM 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 3 **Store ≤ -20°C**
- 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°C. The calibrator stock should be stored at or below -20°C. 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 IgM levels in rat serum or plasma collected 5 days after immunization with TT at which point the immune response originates predominately from IgM.
3. All reagents should be allowed to reach room temperature (18-25°C) before use.
4. The optimal sample dilution should be determined empirically. However, studies suggest an initial sample dilution of 50 fold. It is recommended that samples not be tested at dilutions below 20 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 or deionized water.

### Calibrator

1. The rat anti-tetanus toxoid IgM calibrator is provided as a lyophilized stock. Reconstitute one vial with distilled or deionized water as described on the vial label and mix gently until dissolved. (The reconstituted calibrator is stable for at least one day if stored at 4 °C).
2. Label 6 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25, and 3.125 u/mL.
3. Into the tube labelled 100 u/mL, pipette the volume of diluent indicated on the stock vial label. Then add the volume of reconstituted calibrator stock detailed on the vial label and mix. This provides the 100 u/mL calibrator.
4. Pipette 250  $\mu$ L of diluent into the tubes labelled 50, 25, 12.5, 6.25, and 3.125 u/mL.
5. Into the tube labelled 50 u/mL pipette and mix 250  $\mu$ L of the 100 u/mL reconstituted calibrator. This provides the 50 u/mL calibrator.
6. 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 IgM is present in rat serum or plasma at concentrations of approximately 2,500 u/mL 5 days after immunization with TT. In order to obtain values within range of the calibration curve, we suggest samples initially be diluted 50 fold using the following procedure for each sample tested. Optimal dilutions may need to be determined empirically.

1. For each test sample dispense 343  $\mu$ L diluent into tubes.
2. Pipette and mix 7  $\mu$ L of the serum/plasma sample into the tube containing 343  $\mu$ L of diluent. This provides a 50 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  $\mu$ L of calibrators, and diluted samples into the wells (we recommend that samples be tested in triplicate).
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 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 IgM in u/mL from the calibration curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration for anti-TT 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 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**

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