

Rat Anti-Tetanus Toxoid IgM ELISA Kit

Life Diagnostics, Inc., Catalog Number: 4300-2

ELISA for the Quantitative Determination of Rat Anti-Tetanus Toxoid IgM

INTRODUCTION

Evaluation of the levels of anti-tetanus toxoid IgM after immunization with tetanus toxoid provides a useful indicator of aspects of the immune response. The rat anti-tetanus toxoid IgM ELISA developed by Life Diagnostics, Inc., facilitates rapid and quantitative measurement of rat anti-tetanus toxoid IgM levels in serum or plasma samples.

PRINCIPLE OF THE TEST

The rat anti-tetanus toxoid 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. Standards and diluted serum or plasma samples are 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-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. Anti-tetanus toxoid IgM levels in the samples are derived by reference to a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Tetanus toxoid coated 96-well plate (12 strips of 8 wells)
- Enzyme Conjugate Reagent, 11 ml
- Standard stock¹ (lyophilized), 3 vials **Store ≤ -20°C**
- 20x Wash Solution, 50 ml
- Diluent (30 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized 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 450nm
- Graph paper (PC graphing software is optional)

¹ The levels of rat anti-tetanus toxoid IgM are measured in nominal units and are calibrated with reference anti-tetanus toxoid rat serum at Life Diagnostics, Inc.

STORAGE OF TEST KIT

The reference standard stocks should be stored at or below -20°C. All other kit components should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with 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 rats 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 performed at Life Diagnostics, Inc., using serum obtained from rats immunized intraperitoneally with tetanus toxoid, indicate that an initial sample dilution of 50 fold is a good starting point. **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 the wells of the microtiter plate within 5 minutes.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The rat anti-tetanus toxoid IgM standard 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 standard is stable for at least 1 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 labeled 100 u/ml, pipette the volume of diluent indicated on the stock vial label. Then add the volume of reconstituted standard stock detailed on the vial label and mix. This provides the working 100 u/ml standard.
4. Pipette 250 µl of diluent into the tubes labeled 50, 25, 12.5, 6.25 and 3.125 u/ml.
5. Into the tube labeled 50 u/ml, pipette and mix 250 µl of the reconstituted 100 u/ml anti-tetanus toxoid IgM standard. This provides the 50 u/ml standard.
6. Similarly prepare the 25, 12.5, 6.25 and 3.125 u/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: Studies at Life Diagnostics, Inc., indicate that anti-tetanus toxoid IgM is present in rat serum at concentrations of ~2500 u/ml 5-days after i.p. immunization with tetanus toxoid². We suggest that samples be diluted 50 fold using the following procedure for each sample to be tested.

1. Dispense 343 μ l of diluent into polypropylene or glass tubes.
2. Pipette and mix 7 μ l of the serum/plasma sample into the tube containing 343 μ l of diluent. This provides a 50 fold diluted sample.
3. Repeat this procedure for each sample to be tested

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder (standards should be tested in duplicate and we recommend that samples be tested in triplicate).
2. Dispense 100 μ l of standards (100 – 6.25 u/ml) and diluted samples into appropriate wells.
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 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 (18-25°C) 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 that 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 (A_{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard 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-tetanus toxoid IgM in u/ml from the standard 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.

² Please note that the levels of anti-tetanus toxoid IgM in a particular study can vary significantly depending on the source of tetanus toxoid used for immunization. Optimal sample dilutions should therefore be determined empirically.

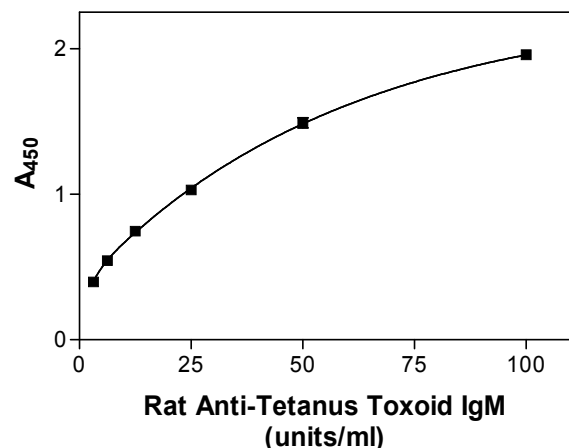
5. PC graphing software may be used for the above steps.
6. If the OD_{450} values of fall outside the standard curve samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against anti-tetanus toxoid IgM concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

| Anti-tetanus toxoid IgM (u/ml) | Absorbance (450 nm) |
|--------------------------------|---------------------|
| 100 | 1.956 |
| 50 | 1.491 |
| 25 | 1.029 |
| 12.5 | 0.745 |
| 6.25 | 0.543 |
| 3.125 | 0.396 |

Representative Rat Anti-Tetanus Toxoid IgM Standard Curve



LIMITATIONS OF THE PROCEDURE

1. 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.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.