Version 27-04/25

User's Manual

Rat Tetanus Toxoid IgM ELISA Kit

REF **DEIASL076**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This test kit allows rapid and quantitative measurement of rat anti-tetanus toxoid IgM levels in serum or plasma samples

General Description

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 test kit developed by Creative Diagnostics, Inc. facilitates rapid and quantitative measurement of rat anti-tetanus toxoid IgM levels in serum or plasma samples.

Principles of Testing

The rat anti-tetanus toxoid IgM test kit 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 1 hour. 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.

Reagents And Materials Provided

1. Tetanus Toxoid Coated 96-well Plate (12 strips of 8 wells)
2. Enzyme Conjugate Reagent, 11 mL
3. Standard Stock(lyophilized), Store < -20°C
4. 20x Wash Solution, 50 mL
5. Diluent, 30 mL
6. TMB Reagent (One-Step), 11 mL
7. Stop Solution (1N HCl), 11 mL

Materials Required But Not Supplied

1. Precision pipettes and tips
2. Distilled or deionized water
3. Polypropylene or glass tubes

4. Vortex mixer
5. Absorbent paper or paper towels
6. Micro-plate incubator/shaker with mixing speed of ~150 rpm
7. Plate washer
8. Plate reader with an optical density range of 0-4 at 450nm
9. Graph paper (PC graphing software is optional)

Storage

1. The reference standard should be stored at -20°C for optimal stability.
2. All remaining kit components should be stored at 2-8°C.
3. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kit will remain stable for six months from the date of purchase provided that the components are stored as described above.

Specimen Collection And Preparation

General Note: Studies at Creative 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 toxoid. We suggest that samples be diluted 50 fold using the following procedure:

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

Reagent Preparation

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 with the volume of diluent indicated on the vial label to give a 200 units/mL (u/mL) solution of rat anti-tetanus toxoid IgM (the reconstituted standard should be aliquoted and frozen at or below -20°C after reconstitution if additional use is intended).
2. Label 6 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25 and 3.125 u/mL, and pipette 250 µL of diluent into each tube.
3. Into the tube labeled 100 u/mL, pipette and mix 250 µL of the 200 u/mL anti-tetanus toxoid IgM standard. This provides the 100 u/mL standard.
4. Similarly prepare the 50, 25, 12.5, 6.25 and 3.125 u/mL standards by serial dilution.

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 µL of standards (200–3.125 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 1 hour.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400µL/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 (18-25°C) 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 that all the blue color changes to yellow.
13. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

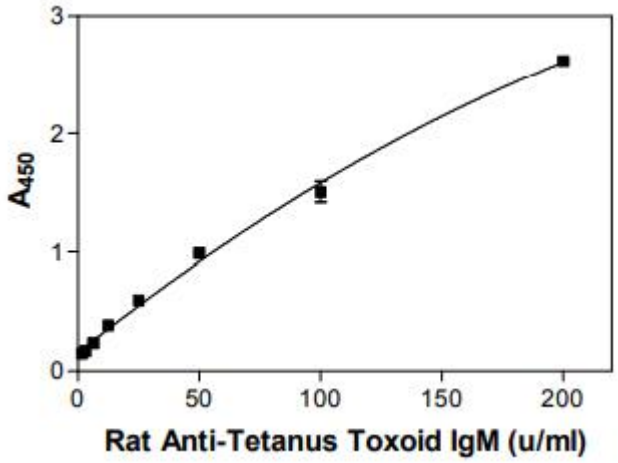
Calculation

1. Calculate the average absorbance values (A450) 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 concentration 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 OD450 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 450 nm 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.

Anti-tetanus toxoid IgM (u/ml)	A_{450}
200	2.769
100	1.646
50	1.029
25	0.571
12.5	0.343
6.25	0.221
3.13	0.143



Precautions

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 Creative 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. Optimal results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

Limitations

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.