Version 23-04/25

User's Manual

Rat Tetanus Toxoid IgG ELISA Kit

REF **DEIASL075**

Σ **96T**

RUO

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 IgG levels in serum or plasma samples.

General Description

Evaluation of the levels of anti-tetanus toxoid IgG following immunization with tetanus toxoid provides a useful indicator of aspects of the immune response. The rat anti-tetanus toxoid IgG test kit developed by Creative Diagnostics, Inc. facilitates rapid and quantitative measurement of rat anti-tetanus toxoid IgG levels in serum or plasma samples.

Principles of Testing

The rat anti-tetanus toxoid IgG 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 IgG antibodies for detection. Test serum or plasma samples are diluted and 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 IgG 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 IgG is proportional to the optical density of the test sample.

Reagents And Materials Provided

1. Tetanus Toxoid Coated 96-well Plate (12 strips of 8 wells)
2. Enzyme Conjugate Reagent, 11 mL
3. Reference Standard (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 IgG is present in rat serum at concentrations of 50,000 u/mL or greater. We suggest that samples be diluted 5,000 fold using the following procedure. Optimum dilutions may need to be determined empirically.

1. Dispense 198 µL and 294 µL of diluent into separate polypropylene or glass tubes.
2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 198 µL of diluent. This provides a 100 fold diluted sample.
3. Dilute 6 µL of the 100 fold diluted sample into the tube containing 294 µL of diluent and mix. This provides a 5,000 fold diluted sample.
4. 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 IgG standard is provided as a lyophilized stock. Reconstitute as detailed on the vial label (the reconstituted standard should be aliquoted and frozen at or below -20°C after reconstitution if additional use is intended).
2. Label 8 polypropylene tubes as 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, and 0 units/mL (u/mL).
3. Into the tube labeled 25 u/mL, pipette the volume of diluent indicated on the IgG standard vial label. Then add the volume of IgG standard indicated on the vial label and mix gently. This provides the 25 u/mL standard.

4. Dispense 250 µL of diluent into the tubes labeled 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, and 0 u/mL.
5. Prepare a 12.5 u/mL standard by diluting and mixing 250 µL of the 25 u/mL standard with 250 µL of diluent in the tube labeled 12.5 u/mL.
6. Similarly prepare the 6.25, 3.13, 1.56, 0.78, and 0.39 u/mL standards by serial dilution.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of standards (100–3.125 u/mL) and diluted samples into the wells (standards 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 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

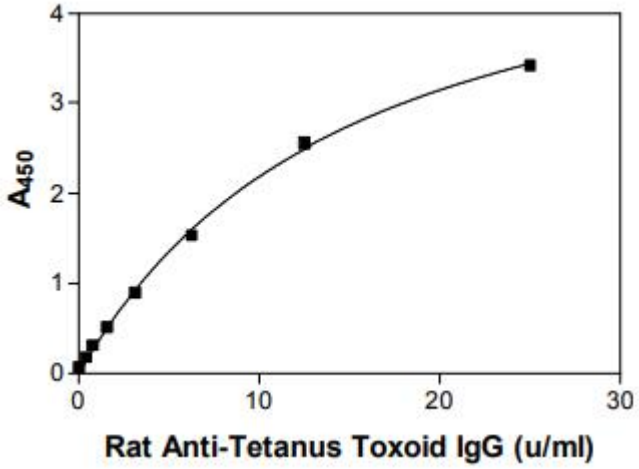
1. Calculate the average absorbance values (A450) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in u/mL on linear graph paper, with absorbance values on the vertical or Y-axis 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 IgG 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 IgG in the serum/plasma sample.
5. PC graphing software may be used for the above steps.
6. If the OD450 values of samples fall outside the standard curve when tested at a 5,000 fold dilution, 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 IgG concentrations on the Xaxis 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 IgG (u/ml)	A_{450}
25	3.403
12.5	2.525
6.25	1.553
3.13	0.903
1.56	0.521
0.78	0.312
0.39	0.177
0	0.065



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 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. However, studies performed at Creative Diagnostics, Inc. using serum obtained from rats immunized intraperitoneally with tetanus toxoid, indicate that an initial sample dilution of 5,000 fold is a good starting point. Do not test samples at dilutions below 200 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.

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