Version 24-04/25

User's Manual

Monkey Tetanus Toxoid IgM ELISA Kit

REF **DEIASL078**

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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 antitetanus toxoid IgM levels in rhesus or cynomolgus monkey serum or plasma.

General Description

Drug candidates are routinely screened for evidence of immune system regulation during the discovery process. It is important that the immune response is not enhanced or decreased since such effects may lead to hypersensitivity or increased susceptibility to infection. Determination of a drug candidate's effects on antitetanus toxoid antibody levels allows easy assessment of immune system regulation. Animals are immunized with tetanus toxoid while undergoing drug treatment and serum is collected at appropriate times post immunization. Serum collected 5-7 days after immunization is used for measurement of anti-tetanus toxoid IgM levels, and serum collected 14+ days post immunization is used to measure anti-tetanus toxoid IgG levels. Comparison of anti-tetanus toxoid IgG or IgM levels in drug treated versus control groups reveals effects on the immune response.

Principles of Testing

The monkey 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 a horseradish peroxidase (HRP) conjugated anti-monkey IgM monoclonal antibody (LDI clone 2C11-1-5) for detection. Standards and diluted serum or plasma samples are incubated in the microtiter wells for 60 minutes. The microtiter wells are subsequently washed, and HRP conjugate is added and incubated for 45 minutes. Antitetanus 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. 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. 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. Anti-Monkey IgM HRP Conjugate, 11 mL
3. Standard Stock. Store < -20°C
4. 20x Wash Solution: TBS50-20, 50 mL
5. Diluent: YD30-1, 30 mL
6. TMB Reagent): TMB11-1, 11 mL
7. Stop Solution (1N HCl): SS11-1, 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 monkey serum at concentrations of 75 µg/mL or greater. We suggest that samples be diluted 1000-fold using the following procedure for each sample to be tested. Dilutions lower than 100-fold should not be tested.

1. Dilute 5 µL of serum with 95 µL of diluent to give a 20-fold dilution.
2. Dilute 10 µL of the 20-fold diluted sample with 490 µL of diluent to give a 1000-fold dilution.

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. Reconstitute the lyophilized anti-tetanus toxoid IgM stock as detailed on the vial label. The reconstituted stock is stable for

one day at 4°C but should be re-frozen if future use is intended.

2. Prepare the 250 ng/mL standard as detailed on the stock vial label.
3. Label 6 micro centrifuge tubes as 125, 62.5, 31.2, 15.63, 7.81 and 3.91 ng/mL and dispense 250 µL of

diluent into each tube.

4. Prepare the 125 ng/mL standard by diluting and mixing 250 µL of the 250 ng/mL standard with 250 µL of diluent in the tube labeled 125 ng/mL.
5. Similarly prepare the remaining standards by two-fold serial dilution.

Assay Procedure

1. Secure the desired number of coated wells in the holder (we suggest that standards and samples be tested in duplicate).
2. Dispense 100 µL of standards 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 60 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°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.

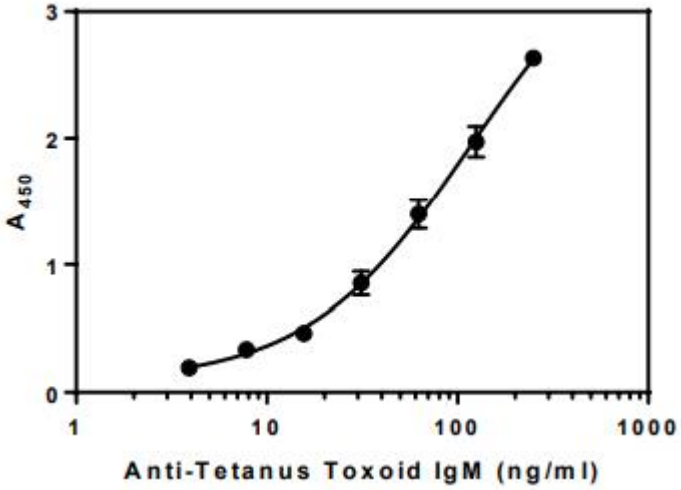
Calculation

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 of each reference standard versus the log10 of the anti-tetanus toxoid IgM concentration.
3. Fit the data using a variable slope, four-parameter logistic curve (x = logx) and derive the concentration of unknowns (remember to derive the anti-log).
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. 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 450nm on the Y-axis against anti-tetanus toxoid IgM concentrations on the Xaxis is shown below. This curve is for 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 (ng/ml)	A_{450}
250	2.638
125	2.059
62.5	1.484
31.25	0.868
15.63	0.495
7.81	0.326
3.91	0.188



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 monkeys 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. indicate that an initial sample dilution of 1000-fold is a good starting point. It is recommended that samples not be tested at dilutions below 400-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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