

Monkey Anti-Tetanus Toxoid IgM ELISA Kit

ELISA for the Quantitative Determination of Monkey 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 monkey anti-tetanus toxoid IgM ELISA facilitates rapid and quantitative measurement of monkey anti-tetanus toxoid IgM levels in serum or plasma samples.

PRINCIPLE OF THE TEST

The monkey 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-monkey IgM antibodies 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. 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), 2 vials **Store \leq -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)

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 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 Life Diagnostics, Inc., using serum obtained from monkeys immunized intraperitoneally with tetanus toxoid, indicate that an initial sample dilution of 2000 fold is a good starting point. **It is recommended that samples not be tested at dilutions below 400 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 monkey 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. Further dilute as described on the vial label to prepare a 100 u/ml working standard (**the reconstituted standard is stable for at least 1 day if stored at 4°C but should be aliquoted and frozen if use beyond that time is intended**).
2. Label 6 polypropylene or glass tubes as 50, 25, 12.5, 6.25 and 3.125 u/ml. Pipette 250 μ l of diluent into each tube.
3. Into the tube labeled 50 u/ml, pipette 250 μ l of the 100 u/ml standard and mix. This provides the 50 u/ml standard.
4. 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 monkey serum at concentrations of ~50,000 u/ml². We suggest that samples be diluted 2000 fold using the following procedure for each sample to be tested.

1. Dispense 95µl of diluent into polypropylene or glass tube.
2. Pipette and mix 5µl of the serum/plasma sample into the tube containing 95 µl of diluent. This provides a 20 fold diluted sample.
3. Dispense 297 ul of diluent into polypropylene or glass tube. Add 3ul of the 20 fold diluted sample and mix for a final dilution of 2000.
4. Repeat this procedure for each sample to be tested.

It is recommended that samples not be tested at dilutions below 400 fold.

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 (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 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 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.

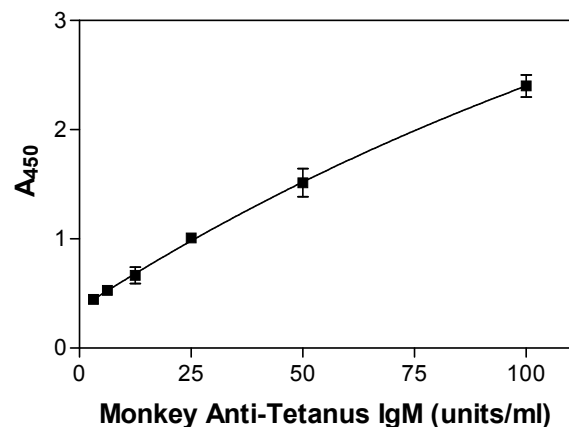
3. Using the mean absorbance value for each sample, determine the corresponding concentration of anti-tetanus toxoid IgM in u/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of anti-tetanus toxoid IgM in the serum/plasma sample.
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	2.401
50	1.515
25	1.010
12.5	0.667
6.25	0.528
3.125	0.448

Typical Monkey 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.

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.