Mouse Anti-Tetanus Toxoid ELISA

REF KBMV005

Ver2.0

RUO

High Sensitive ELISA for Quantitative estimation of Anti-Tetanus Toxoid from Mouse Serum, Plasma.

RUO	For Research Use Only	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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Introduction:

Tetanus is a medical condition characterized by a prolonged contraction of skeletal muscle fibers. The primary symptoms are caused by tetanospasmin, a neurotoxin produced by the Gram-positive, rod-shaped, obligate anaerobic bacterium Clostridium tetani.

Intended Use:

This kit is used to assay the Tetanus Antibody (Tetanus Ab) in the sample of mouse serum and blood plasma.

Principle:

In Mouse Anti-Tetanus Toxoid ELISA, Tetanus Toxoid (TT) is pre-coated in microwell. Addition of Standard or Samples containing Mouse Tetanus Antibody (Anti-TT Ab) reacts with pre-coated Tetanus Toxoid and form a Immune complex in microwell. Followed by addition of HRP Conjugate and incubation, (TT)- (Anti-TT Ab)-(HRP Conjugate) immune complex will be formed, which is directly proportional to the concentration of (Anti-TT Ab) .Upon washing, unbound HRP Conjugate will be removed. TMB substrate is added to the wells. The amount of hydrolyzed substrate is read on a microplate reader and it is directly proportional to the concentration of Tetanus Antibody (Anti-TT Ab) present in standard or sample.

Materials Provided:

1. Microtiter Coated Plate (12 X 8 wells) – 1 no
2. Sample Diluent – 25 ml
3. HRP Conjugate – 12 ml
4. Standard (960 ng/ml) – 0.5 ml
5. Wash Buffer (20X) – 25 ml
6. TMB Substrate – 12 ml
7. Stop Solution – 12 ml
8. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450nm.
2. Adjustable pipettes and pipette tips to measure volumes ranging from 50µl to 1000µl.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Semi log graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:

1. Store all main kit components at 2-8°C.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at temperature < -20°C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at temperature < -20°C. Avoid repeated freeze/thaw cycles.

Reagent Preparation:

1. To make 1X Wash Solution, add 10 ml of 20X Wash Buffer in 190 ml of DI water.

Assay Procedure:

1) Standards Dilution: Prepare the standards as per the table given below using the provided standard Concentration and sample diluent.

240 ng/ml	Standard No.7	125 µl Original Standard + 375 µl Sample diluent
120 ng/ml	Standard No.6	250 µl Standard No.5 + 250 µl Sample diluent
60 ng/ml	Standard No.5	250 µl Standard No.4 + 250 µl Sample diluent
30 ng/ml	Standard No.4	250 µl Standard No.3 + 250 µl Sample diluent
15 ng/ml	Standard No.3	250 µl Standard No.2 + 250 µl Sample diluent
7.5 ng/ml	Standard No.2	250 µl Standard No.3 + 250 µl Sample diluent
3.75 ng/ml	Standard No.1	250 µl Standard No.2 + 250 µl Sample diluent

ALL THE STEPS MUST BE PERFORMED AT 37°C

2) The quantity of the plates depends on the quantities of samples and standards to be tested. It is suggested to duplicate each standard and blank well. Every sample shall be made according to the requirement and try to use the duplicated well as possible.

DETAILED WORKING STEPS

Dispense 100 µl of Standard
Or Dispense 90 µl of Sample Diluent and 10 µl of sample per well.
↓
Cover the plate and incubate for 60 minutes at 37°C.
↓
Decant, then wash each well 5x with 300 µl wash solution (with 30s soak time) and tap dry onto absorbent paper.
↓
Dispense 100 µl of HRP Conjugate per well.
↓
Cover plate and incubate for 60 minutes at 37°C.
↓
Decant, then wash each well 5x with 300 µl wash solution (with 30s soak time) and tap dry onto absorbent paper.
↓
Dispense 100 µl TMB substrate per well.
↓
Incubate for 5-30 minutes at 37°C protected from light.
↓
Dispense 100 µl stop solution per well, mix gently.
↓
Read OD at 450 nm with a microplate reader within 30 minutes after reaction stop.

Calculation of Results:

Determine the mean absorbance for each set of duplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Plot the standard curve on semi log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown sample concentration, find the unknowns mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the unknown sample concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Safety Precautions:

- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.

- Since the kit contains potentially hazardous materials, the following precautions should be observed

- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant

- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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