

GENLISA® Mouse Toxoplasma- Immunoglobulin M, Tox-IgM ELISA

REF : KLM2081

Ver 1.0

RUO

Enzyme Immunoassay for the Qualitative Determination of Toxoplasma-
Immunoglobulin M, Tox-IgM in Mouse serum

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:

The GENLISA® ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs an indirect ELISA technique.

Intended Use:

The GENLISA® Mouse Toxoplasma-Immunoglobulin M, Tox-IgM ELISA kit is used as an analytical tool for qualitative determination of Mouse Toxoplasma-Immunoglobulin M, Tox-IgM in serum.

Principle:

The method employs indirect ELISA technique. Antigens are pre-coated onto microwells. Samples and controls are pipetted into microwells and Mouse Toxoplasma-Immunoglobulin M, Tox-IgM present in the sample are bound by the antigens. Anti-Mouse Antibody IgM conjugated to HRP is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Mouse Toxoplasma-Immunoglobulin M, Tox-IgM in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Mouse Tox-IgM Antibody Microtitre coated plate (8 x 12 wells) - 1 no.
2. Positive Control – 1 ml
3. Negative Control - 1 ml
4. Anti-Mouse Antibody IgM:HRP Conjugate – 12 ml
5. Sample Diluent – 12 ml
6. (20X) Wash Buffer - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Clean tubes and Eppendorf tubes.
6. Precision single and multi-channel pipette and disposable tips.
7. 37°C incubator.
8. Timer.

Handling/Storage:

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Specimen Collection and Preparation:

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- Serum: Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.

Note: Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X)**; dilute **25ml** of **20X Wash Buffer** in **475 ml** of **DI water**.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Mouse Toxoplasma-Immunoglobulin M, Tox-IgM. High Dose Hook Effect is due to excess of antibody for very high concentrations of Mouse Toxoplasma-Immunoglobulin M, Tox-IgM present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent.
3. Mouse Toxoplasma-Immunoglobulin M, Tox-IgM concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN₃), as it could destroy the HRP activity resulting in under-estimation of the amount of Mouse Toxoplasma-Immunoglobulin M, Tox-IgM.
5. It is recommended that all Controls and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Controls and Samples.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that Samples should be run in duplicates.
2. Add **100 ul Negative control and Positive control** to respective control wells except blank wells.
3. Add **100 ul** of **Sample Diluent** to each of the other reaction wells and add **10 ul** of test specimen in order. The color change effect is better when the sample is added and mixed by blowing.
4. Cover the plate with the sealer and incubate for 30 minutes at 37°C.
5. Aspirate and wash plate 5 times with diluted Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
6. Add **100 ul of Anti-Mouse Antibody IgM:HRP Conjugate** to each well except blank well. Mix well.
7. Cover the plate with the sealer and incubate for 30 minutes at 37°C.

8. Aspirate and wash as per step no. 5.
9. Pipette **100 ul TMB Substrate** in each well.
10. Incubate the plate at **37°C** for **15 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
11. Pipette **100 ul of Stop Solution** to all wells. The wells should turn from blue to yellow in color.
12. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Cut Off Value (C.O.) = NCx + 0.10

NCx: Mean Absorbance of Negative Control.

Validity of the test:

Mean of the Positive Control ≥ 0.8

Mean of the Negative Control ≤ 0.1 (If one well OD>0.1, it should be abandoned; If two wells A>0.1, it should be retested)

Interpretation of Results

Negative determinant: if the sample OD value $\leq$ Cut Off, the Mouse Toxoplasma-Immunoglobulin M, Tox-IgM is Negative.

Positive determinant: if the sample OD value $>$ Cut Off, the Mouse Toxoplasma-Immunoglobulin M, Tox-IgM is Positive.

Safety Precautions:

- **This kit is For Research Use only.** Follow the working instructions carefully.
- 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.
- 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.



Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Blank Blank			
1B 2B	Positive Control Positive Control			
1C 2C	Negative Control Negative Control			
1D 2D	Sample Sample			
1E 2E	Sample Sample			
1F 2F	Sample Sample			
1G 2G	Sample Sample			
1H 2H	Sample Sample			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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SYMBOLS KEY

	Coated Microtiter Plate (8 x 12 wells)
	Positive Control
	Negative Control
	HRP- Conjugate
	Sample Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature