Dengue Antibody IgM GENLISA™ ELISA

REF : KBD769

Ver 2.0

IVD

Enzyme Immunoassay for Qualitative Determination of Dengue Antibody IgM in human serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF **KBD768**  **96 tests**

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Introduction:

Dengue virus is a member of the virus family Flaviviridae and is transmitted to people through the bite of the mosquitos Aedes aegypti and Aedes albopictus. Each year, 100 million people become infected with dengue virus. However, the majority of deaths that result from dengue infection result from Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).

The incubation period of dengue fever is approximately four days. It is difficult to distinguish dengue fever from other viral diseases and the person usually recovers in 5 days.DHF has a similar incubation period as dengue fever and many of the same symptoms. However, the fever is more severe and the drowsiness and lethargy is more extreme. This can cause the individual to lose blood volume, result in hypotension, go into shock (DSS) and die.

It is important to understand why an individual will develop DHF/DSS. The Dengue virus has been shown to have 4 subtypes. These 4 subtypes are different strains of dengue virus that have 60-80% homology between each other. After a person is infected with dengue, they develop an immune response to that dengue subtype. The immune response produced specific antibodies to that subtype's surface proteins that prevents the virus from binding to macrophage cells (the target cell that dengue viruses infect) and gaining entry.

Intended Use:

The Dengue Antibody IgM GENLISA™ ELISA is intended for the qualitative determination of Dengue IgM class antibodies in human serum and plasma.

Principle:

Dengue Antibody IgM GENLISA™ ELISA is an indirect enzyme linked immunosorbent assay for qualitative determination of IgM antibody present in the human serum and plasma. Antigens are pre-coated onto microwells. Samples, Controls are pipetted into microwells and Dengue antibody present in sample binds to the antigen coated on the wells. Anti-Human IgM:HRP Conjugate antibody is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of Dengue Antibody IgM present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Dengue Antigen Coated Microtiter Plate (12 x 8 wells) – 1 no
2. Negative Control – 2 ml
3. Positive Control – 2 ml
4. Enzyme Conjugate Antibody - 12 ml
5. (20X) Wash Buffer – 50 ml
6. Sample Diluent – 2 x 50 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Neutralizing Reagent – 6.5 ml
10. 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 (1000, 100 and 10 ul)
3. Deionized (DI) water
4. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (+/-0.5°C tolerance)
5. Wash bottle or automated microplate washer

6. Graph paper or software for data analysis
7. Timer
8. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. 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. 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:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, re-centrifuge.

Sample Dilution: To make 1:101 dilution, dilute 10 ul Sample + 1000 ul Sample Diluent and mix thoroughly.

Reagent Preparation:

1. To make Wash Buffer (1X); dilute 25 ml of 20X Wash Buffer in 475 ml of DI water. This is the working solution.

2. Allow all components to reach RT (Room Temperature) prior to use in the assay.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.

2. Add **50 ul** of **Neutralizing Reagent** to each well except the blank well.

3. Add **100 ul Positive, Negative Controls and Diluted Sample** to the sample wells.

4. Seal the plate using the sealing membrane. Incubate at 37ºC for 1 hour.

5. Aspirate and wash plate 6 times with **(1X) Wash Buffer** 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. All the washes should be performed similarly.

6. Add **100 ul** of **Enzyme Conjugate Antibody** to each well except the blank well and incubate at 37ºC for 1 hour.

7. Repeat the Wash step 5.

8. Add **100 ul** of **TMB Substrate** into each well except blank well.

9. Incubate at RT for 20 minutes.

10. Add **100 ul** of **Stop Solution**.

11. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Calculation of Results:

If the test turns out to be valid, results are calculated from the mean OD450nm value of the Negative Control (NC) by means of a cut-off value (Co) determined with the following formula:

Cut-Off = NC + 0.250

Interpretation of Results:

Test results are interpreted as a ratio of the sample OD450nm value (S) and the cut-off value (Co), or S/Co, according to the following table:

S/Co	Interpretation	
< 0.9	Negative	Antibodies against the pathogen are present.
0.9 – 1.0	Equivocal	Antibodies against the pathogen could not be detected clearly.
> 1.0	Positive	No antibodies present against specific pathogen.

Reference Values:

It is recommended that each laboratory establishes its own normal and pathological reference ranges, asusually done for other diagnostic parameters, too.

Limitations of Method:

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Performance Characteristics:

Sensitivity
The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is > 98%.

Specificity
The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is > 98%.

Safety Precautions:

- **This kit is For In-vitro Diagnostic 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.
- 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.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- 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

LIMITED WARRANTY

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not applyto product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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THANK YOU FOR USING KRISHGEN PRODUCT!

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **50 ul** of **Neutralizing Reagent** to each well except the blank well.
3	Add **100 ul Controls and Diluted Sample** to the sample wells.
4	Seal the plate using the sealing membrane. Incubate at 37ºC for 1 Hour.
5	Aspirate and wash plate 6 times with **(1X) Wash Buffer** 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. All the washes should be performed similarly
6	Add **100 ul** of **Enzyme Conjugate Antibody** to each well except the blank well and incubate at 37ºC for 1 hour.
7	Repeat the Wash step 4.
8	Add **100 ul** of **TMB Substrate** into each well except blank well
9	Incubate at RT for 20 minutes.
10	Add **100 ul** of **Stop Solution**.
11	Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

SYMBOLS KEY

MTP	Coated Microtiter Plate (12 x 8 wells)
CNTRL	Control
ENZY CONJ	Enzyme Conjugate
SAMP DIL	Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature