






# Chikungunya Antibody IgM GENLISA™ ELISA

**REF** : KBD762

Ver 1.2

**IVD**

Enzyme Immunoassay for Qualitative Determination of Chikungunya IgM antibody in human serum and plasma.

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

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**REF** KBD762

 96 tests

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**Introduction:**

Chikungunya disease is caused by chikungunya virus (CHIKV), a mosquito-borne alphavirus belonging to the family *Togaviridae*. It is an enveloped, positive strand RNA virus, genome consisting of five structural proteins. The two main competent vectors of CHIKV are considered to be *Aedes aegypti* and *Aedes albopictus*. Transmission of virus occurs mainly through the bite of an infected *Aedes* (*subgenus* *Stegomyia*). The virus replicates in the skin, in fibroblasts, and disseminates to the liver, muscle, joints, lymphoid tissue and brain in the human body. The incubation period is 2–4 days and is followed by a sudden onset of clinical disease with no prodromal phase. Symptoms of CHIKV infection include high fever, headache, photophobia and a petechial rash or maculopapular rash. Also, maternal-fetal transmission can occur intrapartum, which results in high rates of infant morbidity.

**Intended Use:**

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

**Principle:**

Chikungunya Antibody IgM GENLISA™ ELISA is an indirect enzyme linked immunosorbent assay for qualitative determination of IgM antibody present in the human serum and plasma. Chikungunya antigens are pre-coated onto microwells. Samples, Controls are pipetted into microwells and Chikungunya IgM antibody present in test sample binds to the antigen coated on the wells. After washing, anti-human IgM HRP conjugate is added 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 Chikungunya IgM antibody present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

**Materials Provided:**

1. Chikungunya Antigen Coated Microtiter Plate (8x12 wells) – 1 no
2. Negative Control – 2 ml
3. Positive Control – 2 ml
4. Anti-Human IgM:HRP Conjugate - 12 ml
5. (20X) Wash Buffer - 25 ml
6. Sample Diluent - 50 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 5 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. 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 minutes at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

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

**Sample Dilution:**

To make 1:100 Dilution, dilute 5 ul Sample + 495 ul Sample Diluent. Mix well.

**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 **100 ul Controls**, diluted **Sample** in appropriate wells.
3. Seal the plate and Incubate at 37°C for 60 minutes.
4. Aspirate and wash plate 5 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.
5. Add **100 ul of Anti-Human IgM:HRP Conjugate** to each well. Incubate at 37°C for 30 minutes.
6. Repeat Wash Step as mentioned in step 4.
7. Add **100 ul of TMB Substrate** into each well.
8. Incubate at RT for 30 minutes.
9. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

**Results Calculation:****Cut Off Value (CO) Calculation:**

**Cut Off (CO) = Mean O.D of Neg. Control + 0.2 (Cut Off Factor)**

Example:

$$\begin{aligned}\text{Cut Off Value} &= \text{O.D. of Neg. Control 1} + \text{O.D. of Neg. Control 2} / 2 + 0.2 \\ &= 0.102 + 0.132 / 2 + 0.2 \\ &= 0.317\end{aligned}$$

**Reference Values:**

**Negative Results:** Samples with O.D below or equal to the Cut Off Value (COV) are reported as Non-Reactive.

**Equivocal Results:** Samples with O.D above Cut Off Value (COV) and below or equal to O.D of 0.5 are reported as Equivocal

**Positive Results:** Samples with O.D above 0.5 are reported as Reactive.

**Interpretation of Results:**

<b>Negative Value</b>	Absorbance $\leq$ COV	No antibodies present against specific pathogen.
<b>Equivocal*</b>	Absorbance $>$ COV and $\leq$ 0.5	Equivocal Samples should be retested.
<b>Positive Value</b>	Absorbance $>$ 0.5	Antibodies against specific pathogen are present.

**Criteria of Validation:**

Chikungunya Virus Antibody IgM results are considered to be valid, if

<b>Negative Control</b>	O.D $<$ 0.2
<b>Positive Control</b>	O.D $>$ 0.5

**State Of Infection:**

<b>Serology</b>	<b>Significance</b>
IgM	Primary Antibody Response High IgM titer with Low IgG titer: Current or recent Infection. Persisting IgM: Rare.
IgG	Secondary Antibody Response. May persist for several years. High IgG titer with Low IgM titer: Indicates Past Infection.

**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 of the Kit:**

This kit has been developed and validated as per regulatory guidelines.

**Sensitivity:**

10 known Chikungunya Virus Antibody IgM positive patient serum samples were tested using Chikungunya Virus Antibody IgM GENLISA™ ELISA and all the 10 samples tested positive. 100% sensitivity was observed.

**Specificity:**

20 known Chikungunya Virus Antibody IgM negative patient serum samples were tested using Chikungunya Virus Antibody IgM GENLISA™ ELISA and all the 20 samples tested negative. 100% specificity was observed

**Clinical Sensitivity/Specificity:**

Total number of 10 known Chikungunya Virus Antibody IgM positive patient serum samples and 20 negative patient serum samples were run using the Chikungunya Virus Antibody IgM GENLISA™ ELISA. The results were correlated as under –

Particulars	Specificity as per Chikungunya Virus Antibody IgM GENLISA™ ELISA	%
n=10 Positive Patient Samples	10	100
n=20 Negative Patient Samples	20	100

**Precision:**

Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low, medium and high concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<10%	<12%
Medium	<10%	<10%
High	<10%	<10%

**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 apply to 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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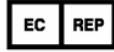
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**THANK YOU FOR USING KRISHGEN PRODUCT!**



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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 <b>100 ul Controls, Diluted Sample</b> in appropriate wells.
3	Seal the plate and <b>Incubate at 37°C for 60 minutes.</b>
4	Aspirate and wash plate 5 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.
5	Add <b>100 ul of Anti-Human IgM:HRP Conjugate</b> to each well except blank well. <b>Incubate at 37°C for 30 minutes.</b>
6	Repeat the Wash Step as mentioned in step 4.
7	Add <b>100 ul of TMB Substrate</b> into each well.
8	<b>Incubate at RT for 30 minutes.</b>
9	Add <b>100 ul of Stop Solution.</b> Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

## SYMBOLS KEY

<b>MTP</b>	Coated Microtiter Plate (8x12 wells)
<b>CNTRL</b>	Control
<b>ENZY CONJ</b>	Enzyme Conjugate
<b>SAMP DIL</b>	Sample Diluent
<b>20x WASH BUF</b>	(20x) Wash Buffer
<b>SUB TMB</b>	TMB Substrate
<b>SOLN STOP</b>	Stop Solution
	Consult Instructions for Use
<b>REF</b>	Catalog Number
	Expiration Date
	Storage Temperature