Mumps IgM GENLISA™ ELISA

REF : KBD766

Ver 1.1

IVD

Enzyme Immunoassay for Qualitative Determination of Mumps 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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CE **REF** KBD766 Σ 96 tests

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Introduction:

Mumps Virus (MuV) is the member of the genus *Rubulavirus* in the *Paramyxoviridae* family. The MuV genome is a non-segmented single-stranded negative RNA. It is a type of acute respiratory infectious disease that is prevalent worldwide. The inflammation and swelling of the parotid glands are the main clinical features of MuV infection, but the virus can also injure many internal organs and the CNS and can cause the emergence of a variety of clinical manifestations, including pancreatitis, orchitis, deafness, sterile meningitis, encephalitis, and other complications. It is transmitted through respiratory droplets. The Median incubation period is 19 days ranging (15-24 days), with a serial interval of around 20 days. It can be isolated from 7 days before to 9 days after onset of symptoms.

Intended Use:

The Mumps IgM GENLISA™ ELISA is intended for the qualitative determination of IgMclass antibodies in human serum and plasma.

Principle:

Mumps IgM GENLISA™ ELISA is an indirect enzyme linked immnunosorbent assay for qualitative determination of Mumps IgM antibody present in the human serum and plasma. Antigens are pre-coated onto microwells. Samples and Controls are pipetted into microwells and Mumps IgM present in test sample binds to the antigen coated on the wells. And then enzyme 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 Mumps Antibody present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Recombinant Mumps Coated Microtiter Plate (96 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 - 30 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 2 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, add 3 ul Sample + 300 ul Sample Diluent

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 30 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 the Wash Step as mentioned in step 4.
7. Add **100 ul** of **TMB Substrate** into each well.
8. Incubate at 37°C 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.

Criteria of Validation:

Negative Control	O.D < 0.2
Positive Control	O.D > 0.5

Calculation:

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

Example:

Cut Off Value = O.D. of Neg. Control 1 + O.D. of Neg. Control 2 / 2 + 0.2
=0.098 + 0.112/2 +0.2
= 0.305

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:

Negative Value	Absorbance ≤ COV	No antibodies present against specific pathogen.
Equivocal*	Absorbance > COV and ≤ 0.5	Equivocal Samples should be retested.
Positive Value	Absorbance > 0.5	Antibodies against specific pathogen are present.

Criteria of Validation:

Mumps Virus Antibody IgM results are considered to be valid, if
O.D. of the Negative Control ≤ 0.2
O.D. of the Positive Control > 0.5

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:
5 known Mumps Virus Antibody IgM positive patient serum samples were tested using Mumps Virus Antibody IgM GENLISA™ ELISA and all the 5 samples tested positive. 100% sensitivity was observed.

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

Clinical Sensitivity/Specificity:
Total number of 5 known Mumps Virus Antibody IgM positive patient serum samples and 20 negative patient serum samples were run using the Mumps Virus Antibody IgM GENLISA™ ELISA. The results were correlated as under -

Particulars	Specificty as per Mumps Virus Antibody IgM GENLISA™ ELISA	%
n=5 Positive Patient Samples	5	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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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 **100 ul Controls, Diluted Sample** in appropriate wells
3	Seal the plate and Incubate at 37°C for 30 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 Enzyme Conjugate** to each well except blank well.
6	Incubate at 37°C for 30 minutes.
7	Repeat the Wash Step as mentioned in step 4.
8	Add **100 ul** of **TMB Substrate** into each well.
9	**Incubate** at **37°C** for **30 minutes**.
10	Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction.

SYMBOLS KEY

MTP	Coated Microtiter Plate (8 x 12 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