KRISHGEN PUDGALA
PARTNER FOR PRECISION DIAGNOSTICS

Mumps IgG GENLISA™ ELISA

REF : KBD765

Ver 1.2

IVD

Enzyme Immunoassay for Qualitative Determination of Mumps Antibody IgG 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**

CE

REF KBD765

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 IgG GENLISA™ ELISA is intended for the qualitative determination of IgG class antibodies in human serum and plasma.

Principle:

Mumps IgG GENLISA™ ELISA is an indirect enzyme linked immnunosorbent assay for qualitative determination of Mumps IgG antibody present in the human serum and plasma. Antigens are pre-coated onto microwells. Samples and Controls are pipetted into microwells and Mumps IgG 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 (12 x 8 wells) - 1 no
2. Negative Control (ready to use) - 0.5 ml
3. Positive Control (concentrated) - 50 ul
4. Anti-Human IgG:HRP Conjugate - 12 ml
5. Positive Control Diluent – 3 ml
6. (20X) Wash Buffer - 25 ml
7. Sample Diluent - 30 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 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 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. Upon receipt it is recommended to immediately make **aliquots of the Positive Control** as per requirement and **freeze at -20°C for long term storage**. For using the same in an assay, bring the aliquot to room temperature and prepare dilution as shown herein below.
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. 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 the sample.

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, re centrifuge.

Sample Dilution:
To make 1:100 Dilution, dilute 5 ul Sample + 495 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. **Positive Control preparation – Dilute the concentrated positive control i.e. 11 ul Positive Control (concentrated) + 99 ul of Positive Control Diluent to prepare 110 ul of Positive Control solution**.
3. Allow all components to reach RT (Room Temperature) prior to use in the assay.

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 Mumps IgG. High Dose Hook Effect is due to excess of antibody for very high concentrations of Mumps IgG present in the sample.
3. Avoid assay of Samples containing sodium azide (NaN3), as it could destroy the HRP activity resulting in under-estimation of the amount of Mumps IgG.
4. It is recommended that all Controls and Samples be assayed in duplicates or triplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. 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.
7. The plates should be read within 15 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Controls and Samples

Test Procedure:

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

2. Add **100 ul Negative Controls, Positive Control solution (prepared), 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 IgG: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 **RT for 30 minutes** in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

9. Add **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

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

Calculation of Results:

Method 1

Calculate the cut-off manually to ensure each laboratory assay variance is accommodated. If you wish to run and interpret the results directly, you may use the Method 2 indicated herein below. Method 1 helps ensure the variation in the assays on personnel to personnel basis and on the laboratory floor level are incorporated for a higher degree of accuracy.

Cut Off (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
= 0.102 + 0.132 / 2 + 0.2
= 0.317

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 / Mildly Positive
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 / Mildly Positive	Absorbance > COV and ≤ 0.5	Equivocal Samples should be retested.
Positive Value	Absorbance > 0.5	Antibodies against specific pathogen are present.

Method 2

Alternately, the absorbance values calculated maybe used directly to interpret the results as under -

Cut-Off (CO) = 0.300

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 / Mildly Positive
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 / Mildly Positive	Absorbance > COV and ≤ 0.5	Equivocal Samples should be retested.
Positive Value	Absorbance > 0.5	Antibodies against specific pathogen are present.

Criteria of Validation:

Calculate the cut-off manually to ensure each laboratory assay variance is accommodated. If you wish to run and interpret the results directly, you may use the Method 2 indicated herein below. Method 1 helps ensure the variation in the assays on personnel to personnel basis and on the laboratory floor level are incorporated for a higher degree of accuracy.

Mumps IgG results are considered to be valid, if

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

State Of Infection:

Serology	Significance
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.

Reference Values:

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually 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 of the Kit:

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

Sensitivity:
20 known Mumps IgG positive patient serum samples were tested using Mumps IgG GENLISA™ ELISA and all the 20 samples tested positive. 100% correlation was observed. The control has been calibrated using Human Mumps IgG Plasma (Catalog No. PLS102G) from Serion Diagnostics, Germany and tested positive for reactivity above the cut-off.

Specificity:
5 known Mumps IgG negative patient serum samples were tested using Mumps IgG GENLISA™ ELISA and all the 5 samples tested negative. 100% correlation was observed

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

Particulars	Specificity as per Mumps IgG GENLISA™ ELISA	%
n=20 Positive Patient Samples	20	100
n=5 Negative Patient Samples	5	100

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.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Pudgala LLP shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Pudgala LLP shall not have any other obligation with respect to the products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Pudgala LLP be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Pudgala LLP with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

THANK YOU FOR USING KRISHGEN PRODUCT!

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EC REP

KinesisDx, Lyoner Strasse 14, Frankfurt, Germany

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 Negative Controls, Positive Control solution (prepared), 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 Anti-Human IgG:HRP Conjugate** to each 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 **RT** 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 (12 x 8 wells)**
HRP CONJ	**HRP Conjugate**
SAMP DIL	**Sample Diluent**
PC	**Positive Control**
NC	**Negative Control**
PC DIL	**Positive Control 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**