Mumps IgG GENLISA™ ELISA

REF : KBD765

Ver 1.1

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**

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EC REP **KinesisDx, Lyoner Strasse 14, Frankfurt, Germany**

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 (8 x 12 wells) - 1 no
2. Negative Control - 2 ml
3. Positive Control - 2 ml
4. Anti-Human IgG: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.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm
   Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Sample Dilution:

To make 1:100 Dilution, add 3 ul Sample + 300 ul Sample Diluent

Test Sample Preparation - Samples have to be diluted 1:100 (v/v), e.g. 3 ul sample + 300 ul Sample Diluent prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -20°C. Note: Diluted samples should not be stored as they may show degradation in results.

Reagent Preparation:

1. To make (1X) Wash Buffer; 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.

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 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 IgG:HRP Conjugate** to each well. Incubate at 37°C for 30 minutes.

6. Repeat the Wash Step as mentioned in step (4) above.

7. Add **100 ul** of **TMB Substrate** into each well.

8. Incubate at 37°C 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:

Determine the Mean Absorbance for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off value. The cut-off value is calculated as the mean of absorbance of the Negative Control plus 0.2.
Cut Off (CO) = Mean O.D. of Neg. Control + 0.02 (Cut Off Factor)
Example:
Cut Off Value = O.D. of Neg. Control 1 + O.D. of Neg. Control 2 / 2 + 0.02
= 0.098 + 0.112 / 2 + 0.02
= 0.125

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 0.3 are reported as Equivocal. To be interpreted post retesting with clinical symptoms of the patient.
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 ≤ 0.3	Equivocal Samples should be retested.
Positive Value	Absorbance > 0.3	Antibodies against specific pathogen are present.

Criteria of Validation:

Mumps Antibody IgG results are considered to be valid, if
O.D. of the Negative Control ≤ 0.110
O.D. of the Positive Control > 0.110

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

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.

Note:

All materials including controls have been tested and classified on donor level to be negative or non-reactive for STS, HBsAg, HIV 1 Ag (or HIV PCR (NAT), HCV antibody, HCV (NAT) and HIV 1/2 antibody, as required at the time of bleeding, by using FDA / CDSCO licensed test kits. However, no test system can guarantee complete absence of infectious agents. Therefore, all products should be considered potentially infectious and handled with appropriate safety precautions.

LIMITED WARRANTY

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Krishgen Pudgala LLP, 2023

THANK YOU FOR USING KRISHGEN PRODUCT!

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Regulatory Status:

CE Marked	Europe
FDA registered *	USA
CDSCO registered	India

** under registration. Site establishment undertaken.*

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **100 ul Controls, diluted Samples** 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 except blank well.
6	Incubate at 37°C for 30 minutes.
7	Repeat the Wash Step as mentioned in step (4) above
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