Varicella Zoster Virus (VZV) Antibody IgM GENLISA™ ELISA

REF : KBD981

Ver 1.1

IVD

ELISA immunoassay for qualitative determination of Varicella Zoster Virus (VZV) Antibody IgM in serum and plasma.

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

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REF KBD981 Σ 96 tests

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Introduction:

Varicella-Zoster Virus (human herpes virus 3, HHV-3) belongs to the a-subfamily of herpes viridae. The virus particles measure about 145 nm in diameter. They consist of double stranded DNA, are surrounded by an icosahedral protein capsid and an envelope which contains both host cells and viral components. The virus is usually transmitted in respiratory secretions, and a single serotype causes varicella (Chickenpox), a highly infectious childhood disease, and zoster (shingles), a neurodermic disease; both diseases are found world-wide. Varicella is the acute disease which follows primary contact with the virus, whereas zoster is the response of the partially immune host to a reactivation of the varicella virus present in the body in latent form. Varicella is endemic, most commonly affected are children between 2 and 6 years of age. The course of disease is usually mild and complicated only in immunocompromised children. Rare fatal cases show multiple necrotic lesions in brain, lung (varicella pneumonia), kidneys (hemorrhagic nephritis), spleen, bone marrow, and occasionally in the intestinal tract. The lethality of varicella is below 0.1%. In the infrequent adult infections the disease is more severe, and complications are to be expected in about 5% of all cases. Zoster is of low incidence and appears with increasing frequency and severity with advancing age. Usually the process remains localized; generalization is frequently encountered in a state of immunosuppression. Fatal cases are very rare and nearly always caused by an underlying disease.

Species	Disease	Symptoms (e.g.)	Transmission Route
Varicella-Zoster Virus (VZV)	Varicella (primary infection)	Fever, itching exanthema and vesicular eruptions Complication: pneumonia, inflammations of the brain or bacterial infections. VZV can cause malformations in infants and abortions if the mother has a primary infection in the first trimester of pregnancy	Transmission by droplet; Vertically transmitted infection
	Herpes Zoster (Reactivation)	Fever, malaise, and gastrointestinal disturbance, painful cutaneous vesicular eruption, rash usual unilateral. Complication: Postherpetic neuralgia, infection of the eye, ear, and throat may occur if ophthalmic, trigeminal, or geniculate ganglia are involved; Ramsay-Hunt syndrome.	

Infection or presence of pathogen may be identified by:

- Microscopy
- PCR
- Serology: Detection of antibody production by ELISA

Intended Use:

The Varicella-Zoster Virus (VZV) IgM ELISA is intended for the qualitative determination of IgM class antibodies against Varicella-Zoster Virus (VZV) in human serum or plasma (citrate, heparin).

Principle:

The GENLISA™ Varicella Zoster Antibody IgM ELISA is an enzyme linked immunosorbent assay for qualitative determination of IgM antibody present in the human serum and plasma. VZV antigen is pre-coated onto microwells. Samples, Controls are pipetted into microwells and VZV IgM present in test sample binds to the antigen coated on the wells. After washing, conjugated HRP is added and binds to the captured sample antibody complex. Then, the TMB substrate solution is added to microwells and color develops proportionally to the amount of VZV IgM present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated Plate (12 x 8 wells) - 1 no
2. Sample Dilution Buffer – 100 ml
3. Positive Control - 2 ml
4. Negative Control - 2 ml
5. Cut-Off Control – 3 ml
6. HRP Conjugate - 22 ml

7. (20X) Wash Buffer – 2 x 25 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. Incubator 37°C
3. Adjustable pipettes and multichannel pipettor to measure volumes ranging 10 and 1000 ul
4. Deionized (DI) water
5. Wash bottle or automated microplate washer
6. Graph paper or software for data analysis
7. Timer
8. Absorbent Paper
9. Vortex tube mixer
10. Distilled water
11. Disposable tubes

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, centrifuge again.

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, centrifuge again.

Test Sample Dilution:
All samples should be diluted 1:100 with Sample Dilution buffer. For eg. Dispense 10ul sample and 1ml Sample Dilution Buffer

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. (1X) Wash Buffer Dilution: To make (1X) Wash Buffer, add 25 ml of (20X) Wash Buffer to 475 ml of DI water. This is the working solution.

Test Procedure:

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

2. Add **100 ul Standard, Sample** and **Controls** into respective wells.

3. **Incubate for 1 hour at 37°C.**

4. 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.

5. Add **100 ul** of **HRP Conjugate** to each well except the blank well. Gently mix. Cover the plate using the sealing membrane.

6. Incubate at **room temperature** for 30 minutes.

7. Repeat aspirate and wash step as per (4) above.

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

9. Incubate at **Room Temperature** for 15 minutes in the dark.

10. Add **100 ul** of **Stop Solution** except the blank well, mix well.

11. Read result with an ELISA reader at 450 nm.

Criteria of Validation:

Blank	O.D < 0.100
Negative Control	< 0.2 and < Cut-Off
Cut-Off Control (CO)	O.D between 0.150 – 1.30
Positive Control	O.D >Cut- Off

Calculation of Results:

The Cut-Off is the mean Absorbance Value of the Cut-Off Control determinations.

Example: Absorbance Value Cut-off Control 0.44 + Absorbance Value Cut-off control 0.42
= 0.86 / 2 = 0.43
Cut-Off = 0.43

Results in Units [U]

$$\frac{\text{Sample (mean) Absorbance Value} \times 10}{\text{Cut-off}} = \text{[Bioactiva Units = U]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ U (Units)

Interpretation of Results:

Cut-Off Value	10 U	
Equivocal	9 – 11 U	Antibodies against the specific pathogen cannot be detected clearly.
Negative Value	< 9 U	No antibodies present against specific pathogen.
Positive Value	> 11 U	Antibodies against specific pathogen are present.

Reference Values:

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

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.

Antibody Isotypes and State of Infection:

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection

Performance Characteristics:

Diagnostic Specificity
The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 98.39% (95% confidence interval: 95.92% - 99.56%).

Diagnostic Sensitivity
The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 94.12% (95% confidence interval: 80.32% - 99.28%).

Interferences
Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

Cross Reactivity
Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

Precision:

Intra Assay	N	Mean (E)	CV (%)
#1	24	0.423	11.34
#2	24	0.732	6.87
#3	24	0.514	6.65

Inter Assay	N	Mean (E)	CV (%)
#1	12	15.32	6.89
#2	12	18.67	7.76
#3	12	5.24	11.21

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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Krishgen Pudgala LLP, 2022.

THANK YOU FOR USING A KRISHGEN PRODUCT!

Krishgen Pudgala LLP

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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 Standard, Sample** and **Controls** into respective wells.
3	**Incubate for 1 hour at 37°C.**
4	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
5	Add **100 ul** of **HRP Conjugate** to each well except the blank well. Gently mix. Cover the plate.
6	Incubate at **room temperature** for 30 minutes.
7	Repeat aspirate and wash plate as per Step (4) above.
8	Add **100 ul** of **TMB Substrate** into every well except blank well.
9	Incubate at **Room Temperature** for 15 minutes in the dark
10	Add **100 ul** of **Stop Solution** except the blank well, mix well.
11	Read result with an ELISA reader at 450 nm.

SYMBOLS KEY

MTP	Microtiter Plate (12x8 wells)
PC	Positive Control
NC	Negative Control
Cut-Off CNTRL	Cut Off Control
ENZY CONJ	Enzyme Conjugate
DILN BUF	Dilution Buffer
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