HSV 2 IgM GENLISA™ ELISA

REF : KDT008

Ver 1.2

IVD

Enzyme Immunoassay for the Qualitative Determination of HSV 2 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**

REF KDT008 Σ 96 tests

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Introduction:

Herpes simplex virus (HSV) is the most common human pathogens, human being are the only natural host. The virus exists in the patients, restoring healthy carriers and forms blisters or scars. They also transmit through fluid, saliva and faeces. The main mode of transmission is direct contact, saliva can be contaminated. Indirect transmission can be through other materials e.g. utensils. HSV infection has now become the world's fourth largest infectious diseases.

Herpes simplex virus (HSV) belongs to the herpes virus family subfamily a virus, the virus particle size of about 180 nm. According to difference in the antigenicity of the virus, it is currently divided into type I and type II. Herpes simplex virus is widespread and has many modes of transmission. HSV type I mainly transmits through direct contact with the skin and mucous membrane. HSV type I infection is more common in the population. In pregnant women suffering from primary herpes virus infection, the virus can pass through the placenta causing fetal congenital infection. If a pregnant woman birth canal is infected it can pass to the baby during delivery causing neonatal infection Therefore, HSV type I antibody detection is of great significance to improve the level of eugenics.

Intended Use:

The HSV 2 IgM GENLISA™ ELISA is intended for the qualitative determination of HSV 2 IgM in human serum and plasma.

Principle:

The HSV 2 IgM GENLISA™ ELISA method employs enzyme linked immunosorbent assay (ELISA) technique. Purified anti-human IgM monoclonal antibody are pre-coated onto microwells. Samples and Controls are pipetted into microwells and HSV 2 IgM antibody present in the sample is bound by the antibodies. Enzyme labelled antigen conjugate is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the ready to use TMB substrate solution is added to microwells and color develops proportionally to the amount of HSV 2 IgM antibody present in sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated Plate (8x12 wells) - 1 no
2. Negative Control - 1 ml
3. Positive Control - 1 ml
4. Enzyme Conjugate - 6.5 ml
5. (40X) Wash Buffer - 20 ml
6. Sample Diluent - 11 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 25 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-min 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-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

Reagent Preparation:

1. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 2.5 ml of Wash Buffer (40X) to 97.5 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 Sample Diluent** to the sample wells.
3. Add **10 ul Sample** to the respective sample wells. Mix gently.
4. Dispense **100 ul Negative Control** and **100 ul Positive Control** to the negative and positive wells respectively.
5. Shake gently for 30 seconds to mix well. Incubate at 37ºC for 20 minutes.
6. 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.
7. Add **50 ul** of **Enzyme Conjugate** to each well except the blank well.
8. Incubate at 37ºC for 20 minutes.
9. Repeat the Aspiration / Wash Step.
10. Add **100 ul** of **TMB Substrate** into each well except blank well.
11. Incubate at 37ºC for 10 minutes.
12. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Interpretation of Results:

Determine the Mean Absorbance for each set of duplicate Controls and Samples. Results are interpreted qualitatively by calculating a cut-off value for each sample on the basis of the cut-off determined. Read Absorbance at 450nm with an ELISA reader.

Cut-Off Value (CO) = OD_{mean} of Negative Control x 2.1

Note: Incase Negative Control OD Value is <0.09, the Cut-Off Value is calculated as OD_{mean} of NC (to be taken as) 0.09 x 2.1.
Incase Negative Control OD Value is >0.09, the Cut-Off Value is calculated as OD_{mean} of NC (actual value) x 2.1.

Positive Results: **OD value ≥ CO**
Specimens giving an absorbance equal to or greater than the CO are considered initially reactive, which indicates that Anti-HSV2 antibody IgM has probably been detected using the ELISA.

All initially reactive specimens should be retested in duplicates using the Anti-HSV2 antibody IgM ELISA before the final assay results interpretation. Repeatedly reactive specimens may be considered positive for Anti-HSV2 antibodies with the Anti-HSV2 antibody IgM ELISA.

Negative Results: **OD value < CO**
Specimens giving absorbance less than the CO are negative for the assay, which indicates that no Anti-HSV2 antibody has been detected with the Anti-HSV2 antibody IgM ELISA.

Cut Off Value	OD_{mean} of Negative Control x 2.1
Positive	>= CO
Negative	< CO

Validity of the test:

The test is valid if the following conditions are met,

Mean Absorbance of Negative Control ≤ 0.1
Mean Absorbance of Positive Control ≥ 0.8

Reference Values:

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

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:

Sensitivity:
Limit of Detection: When detecting anti-herpes simplex virus 1 antibody limits, laboratory quality control positive samples diluted till 1:8 with the Anti-HSV2 antibody IgM ELISA kit should be in the positive.

Specificity:
The recombinant antigen used in the kit is specific for Herpes Simplex Virus.

Precision:
Intra-Assay: CV% ≤15%.
Inter-Assay: CV% ≤20%

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.

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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 Sample Diluent** to the **respective sample wells**.
3	Add **10 ul Sample** to the **respective sample wells**. Mix gently.
4	Dispense **100 ul Negative Control** and **100 ul Positive Control** to the negative and positive well respectively.
5	Shake gently for 30 seconds to mix well. **Incubate** at **37ºC** for **20 minutes**.
6	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.
7	Add **50 ul** of **Enzyme Conjugate** to each well except the blank well.
8	Seal the plate and **incubate** at **37ºC** for **20 minutes**.
9	Repeat the Aspiration / Wash Step.
10	Add **100 ul** of **TMB Substrate** to all wells.
11	**Incubate** at **37ºC** for **10 minutes**.
12	Add **100 ul** of **Stop Solution**. **Read result with an ELISA reader at 450 nm** within 15 minutes of stopping the reaction.

SYMBOLS KEY

MTP	Microtiter Plate (8x12 wells)
PC	Positive Control
NC	Negative Control
CONJ	Enzyme Conjugate
SAMP | DIL	Sample Diluent
40X | WASH | BUF	(40X) Wash Buffer
SUB | TMB	TMB Substrate
SOLN | STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature