HSV2 IgM

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IgM antibodies to herpes simplex virus 1(ELISA)

REF: DS180610 Σ **96**

Intended use
The DiaSino HSV2 IgM assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro qualitative determination ofIgM antibodies to Herpes simplex virus 2 (HSV2) in Human serum and plasma.

Summary[1-6]
Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), also known as human herpesvirus 1 and 2 (HHV-1 and HHV-2), are two members of the human Herpesviridae family, a set of viruses that produce viral infections in the majority of humans. Both HSV-1 (which produces most cold sores) and HSV-2 (which produces most genital herpes) are very common and contagious. They can be spread when an infected person begins shedding the virus. About 67% of the world population under the age of 50 has HSV-1. In the United States more than one-in-six people have HSV-2. Although it can be transmitted through any intimate contact, it is one of the most common sexually transmitted infections. HSV evades the immune system through interference with MHC class I antigen presentation on the cell surface, by blocking the transporter associated with antigen processing (TAP) induced by the secretion of ICP-47 by HSV.In the host cell, TAP transports digested viral antigen epitope peptides from the cytosol to the endoplasmic reticulum, allowing these epitopes to be combined with MHC class I molecules and presented on the surface of the cell. Viral epitope presentation with MHC class I is a requirement for activation of cytotoxic T-lymphocytes (CTLs), the major effectors of the cell-mediated immune response against virally-infected cells. ICP-47 prevents initiation of a CTL-response against HSV, allowing the virus to survive for a protracted period in the host.

Test principle
Capture method. Total duration of assay: **70 minutes**.
This kit uses capture ELISA principle to detect HSV-2 IgM. Polystyrene microwell strips are pre-coated with mouse anti-human IgM (anti-μ chain specific). During the first incubation step, all IgM antibodies binds to mouse anti-human IgM monoclonal antibody coated in microplate including the Anti-HSV-2 IgM, the wells are washed to remove unbound serum proteins, and HSV-2 IgM antigens conjugated to the enzyme horseradish peroxidase (HRP-Conjugate) are added. During the second incubation step, if there is HSV-2 IgM antibody in sample, HRP- conjugated antigens will be bound to the anti-human HSV-2 IgM complexes previously formed and the unbound HRP-conjugate is then removed by washing. Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the amount of antibody in the sample respectively.

Reagents
Materials provided
- **Coated Microplate - symbol** HSV2 IgM PLATE 8 x 12 strips, 96 wells.
- **Enzyme Conjugate - symbol** HSV2 IgM CONJ 1 vial, 11.0 mL of HRP (horseradish peroxidase) labeled with Mouse Anti-Human HSV2antigen. Contains 0.1% ProClin300 preservative.
- **Sample Diluent - symbol** HSV2 IgM DILUT 1 vial, 11 mL, ready to use
- **Positive Control - symbol** HSV2 IgM PC 1 vial, 0.2 mL.
- **Negative Control - symbol** HSV2 IgM NC 1 vial, 0.2 mL
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU -** 1 copy.
- **Plate Lid**: 2 pieces.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50 μL with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- For in vitro diagnostic use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Storage and stability
- Store at 2-8°C.
- Seal and return unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum or plasma is recommended for this assay.
- Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 7 days at 2-8°C, and 1 month at -20°C. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate. Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure
Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use.
- **Preparation**: Mark two wells as Negative control (e.g. B1, C1), two wells as Positive control (e.g. D1, E1) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.
- **Adding Sample Diluent:** Add **100 μL** of Sample Diluent into their respective wells except the Blank.
- **Adding Sample**: Add **10 μL** of **Positive control, Negative control,** and **Specimen** into their respective wells except the Blank. Mix by tapping the plate gently. Use a separate disposal pipette tip for each specimen to avoid cross-contamination.
  Note: After adding Sample, the reagents in wells turns **Blue** color from **Green.**
- **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down

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the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Adding Conjugate:** Add **100 µL** of **Conjugate** into each well except the Blank.
- **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer. Each time allow the microwells to soak for **30-60 seconds**. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Coloring:** Add **100 µL** of **Substrate** into each well including the Blank. Incubate the plate at room temperature for 10 minutes avoiding light. The enzymatic reaction between the TMB substrate and the HRP-Conjugate produces blue color in Positive control and in positive sample wells.
- **Stopping:** Using a multichannel pipette or manually, add **50 µL** of **Stop Solution** into each well and mix gently. Intensive yellow color develops in Positive control and HSV2 IgM positive sample wells.
- **Measuring:** Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**.

Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **10 minutes** after stopping the reaction).

Quality control and calculation
- Read the sample's optical density (OD) at 450nm with a micro plate reader.
- Mean negative control OD value ≤ 0.1 and positive control OD value ≥ 0.8, the test is valid, otherwise the test is invalid.
- Cut-Off value (C.O.) = Mean negative control OD value + 0.10 (Calculated by 0.05 when Mean negative control OD value is ≤ 0.05, calculated by actual value when Mean negative control OD value is > 0.05).

Positive Results: Sample O.D value ≥ C.O.
Specimens giving an absorbance equal to or greater than the Cut-Off value are considered initially reactive, which indicates that HSV2 IgM has probably been detected using HSV2 IgM ELISA. All initially reactive specimens should be retested in duplicates using HSV2 IgM ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for HSV2 IgM with HSV2 IgM ELISA.

Negative Results: Sample OD value < C.O
Specimens giving absorbance less than the Cut-Off value are negative for this assay, which indicates that no HSV2 IgM has been detected with HSV2 IgM ELISA, therefore the patient is probably not infected with Toxo and the blood does not contain HSV2 IgM.

Limitations
- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
- Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

Performance Characteristics
Specificity
A study of 118 individuals was tested with DiaSino ELISA kits and found that the specificity was 99.10%.

Sensitivity
Among 137 HSV2 IgM confirmed were positive when tested with this ELISA, and 136 samples of which were detected with positive, the sensitivity was 99.28%.

Precision
Intra-assay
CV ≤ 15%
Within-run precision has been determined by using 15 replicates of three specimens: a low positive, medium positive and a high positive.

Inter-assay
CV ≤ 20%
Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, medium positive and a high positive. Three different lots of the HSV2 IgM ELISA Test Kit have been tested using these specimens over a 5-day period.

Analytical specificity
- Interferences are not observed up to concentrations of 0.1 mg/mL Acetaminophen, 0.2 mg/mL Gentistic Acid, 0.1 mg/mL Ascorbic Acid, 0.1 mg/mL Acetosalisilyc Acid, 0.1 mg/mL Caffeine, 0.6 mg/mL Oxalic Acid, 2 mg/mL Bilirubin, 2 mg/mL Hemoglobin and 1 % Ethanol.
- Rheumatoid factors do not interfere with test .
- Cross-Reactivity are not observed in Syphilis, HBsAg, HCV , HCG positive specimens.

Reference

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3. "Herpes simplex virus". World Health Organization. 31 January 2017.
4. "STD Facts - Genital Herpes". December 11, 2017. Retrieved September 22, 2018. “Genital herpes is common in the United States. More than one out of every six people aged 14 to 49 years have genital herpes.”
5. Straface, Gianluca; Selmin, Alessia; Zanardo, Vincenzo; De Santis, Marco; Ercoli, Alfredo; Scambia, Giovanni (2012). "Herpes Simplex Virus Infection in Pregnancy". Infectious Diseases in Obstetrics and Gynecology.ISSN 1064-7449.
6. Abbas et al (2009) Cellular and Molecular Immunology, Elsevier Inc.

Symbols

IVD **In vitro diagnostic medical device**	8°C / 2°C **Temperature limit**	**Consult instructions for use**	REF **Catalog number**
LOT **Batch code**	**Date of manufacture**	**Use-by date**	Σ **Contains sufficient for <n> tests**
Manufacturer	**Do not use if package is damaged and consult instructions for use**		

diasino
DiaSino Laboratories Co., Ltd
No.68 Jingnansi Road National Eco & Tech Development Area
Zhengzhou 450000, P.R. China. info@diasino.com