Product Information

HSV-1 IgM ELISA kit
Catalog Number: EA100948
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use
The HSV-1 IgM ELISA Kit is intended for the detection of IgM antibody to HSV-1 in human serum or plasma.

Background
HSV-1 and 2 are virtually identical, sharing approximately 50% of their DNA and have over 80% of common antigens. Both types infect the body's mucosal surfaces, usually the mouth or genitals, and then establish latency in the nervous system. For both types, at least two-thirds of infected people have no symptoms, or symptoms too mild to notice. However, both types can recur and spread even when no symptoms are present. By the time they're teenagers or young adults, about 50% of Americans have HSV-1 antibodies in their blood. By the time they are over age 50, some 80-90% of Americans has HSV-1 antibodies. By comparison, almost all HSV-2 is encountered after childhood, when people become sexually active. HSV type 1 is the cause of most orofacial herpes and HSV encephalitis; type 2 is the primary cause of initial and recurrent genital herpes and neonatal HSV. Reactivation of latent HSV infection is a frequent complication of immunosuppression due to cancer, transplantation and AIDS. Asymptomatic genital shedding of HSV-2 is more common than HSV-1 and occurs more frequently during the first 3 months after acquisition of primary type 2 disease than during later periods. The presence of HSV IgG antibody is indicative of previous exposure A significant increases in HSV IgG is an indicative of reactivation, current or recent infection. IgM antibody is present after primary HSV infection.

Principle of the Test
Diluted patient serum (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to wells coated with purified antigen. IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgM specific antibody in the sample.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with HSV-1 antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Calibrator: 1 Vial (ready to use)	1ml

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4. Positive Control: 1 vial (ready to use)	1ml
5. Negative Control: 1 vial (ready to use)	1ml
6. Enzyme conjugate: 1 bottle (ready to use)	12ml
7. TMB Substrate: 1 bottle (ready to use)	12ml
8. Stop Solution: 1 bottle (ready to use)	12ml
9. Wash concentrate 20X: 1 bottle	25ml

Materials and Equipment Required but Not Provided
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel

Disclaimer
This product is for research use only and not intended for diagnostic procedures.

Specimen Collection and Preparation
1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.

Reagent Preparation
1. Prepare 1X Wash buffer by adding Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure
- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl l of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.

7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature. Add 100 µl of stop solution.
8. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm

Calculation of Results
1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of a Standard Curve
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5= 0.400
Positive control O.D. = 1.2
Ab Index = 1.2 / 0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6 / 0.4 = 4.0

Quality Control
The test run may be considered valid provided the following criteria are met:
1. If the O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab index for Positive control should be greater than 1.2.

Interpretation
The following is intended as a guide to interpretation of HSV-1 IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

- **Antibody Index Interpretation**
    - <0.9   No detectable antibody to HSV-1 IgM by ELISA
    - 0.9-1.1   Borderline positive. Follow-up testing is recommended if clinically indicated.
    - >1.1   Detectable antibody to HSV-1 IgM by ELISA

References
1. Langeland N; Haarr L; Mhalu F. Prevalence of HSV-2 antibodies among STD clinic patients in Tanzania. Int J STD AIDS 1998;9(2):104-7.
2. Markoulatos P; Labropoulou V; Kordossi A; Krikelis V; Spyrou N; Moncany ML. A combined indirect ELISA and immunoblotting for the detection of intrathecal herpes simplex virus IgG antibody synthesis in patients with herpes simplex virus encephalitis. J Clin Lab Anal 1995;9(5):325-33.
3. Markoulatos P; Fountoucidou P; Marinakis G; Krikelis V; Spyrou N; Vamvakopoulos N; Moncany ML. Clear detection and typing of herpes simplex virus types 1 and 2 by an indirect ELISA assay: comparison with three different combined methods--capture ELISA, restriction enzymes, and polymerase chain reaction. J Clin Lab Anal 1997; 11(3):146-53.

4. Herbert AM; Bagg J; Walker DM; Davies KJ; Westmoreland D. Seroepidemiology of herpes virus infections among dental personnel. J Dent 1995;23(6):339-42.
5. Goodyear HM; McLeish P; Randall S; Buchan A; Skinner GR; Winther M; Rolland J; Morgan G; Harper JI. Immunological studies of herpes simplex virus infection in children with atopic eczema. Br J Dermatol 1996;134(1):85-93.

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