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Product Information

Mouse/Rat HSV-1 IgG ELISA kit

Catalog Number: EA100947
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Mouse/Rat HSV-1 IgG ELISA test system is an enzyme linked immunosorbent assay (ELISA) is used for the detection of IgG class antibodies to HSV-1 in mouse/rat.

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. Several recent studies have shown the association of more than a dozen herpes viruses with cancer in man and various animals; for example with lymphoma and with squamous cell carcinoma of the lip and cancer of the cervix. 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.The effect of virus dose and animal age on the appearance of acute and latent neurologic infection bu HSV1 and HSV2 was studied in Balb/c and ICR mice inoculated in the footpad. At low viral doses, HSV2 was found to be 1,500 times more neurovirulent than HSV1. The Mp strain of herpes simplex virus type 1 (HSV1) induced a persistent infection in the mouse C 1300 neuronal cell line (clone N 115). C 1300 cultures infected at an MOI of 0.01 or 0.001 survived the initial infection and continued to produce infectious virus and viral antigens for 185 days and 31 days, respectively.

Principle of the Test

Diluted serum is added to wells coated with purified antigen. IgG 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 IgG 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
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 (20-25°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°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.

Limitation of the Test

1. The test results obtained using this kit cannot discriminate between HSV-1 and HSV-2 infection due to the high cross reactivity between the two viruses.
2. Lipemic or hemolyzed samples may cause erroneous results.

References

1. 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.
2. Nahmias AJ, Roizman B. Infection with herpes-simplex viruses 1 and 2. II. N Engl J Med. 1973 289(14):719-25.
3. McKendall RR. Comparative neurovirulence and latency of HSV1 and HSV2 following footpad inoculation in mice. J Med Virol. 1980;5(1):25-32
4. Dawson GJ, Mowshowitz SL, Cohen R, Elizan TS. Herpes simplex virus persistence in mouse neuroblastoma (C 1300) cell cultures: role of interferon. J Neural Transm. 1984; 59(4):309-17.

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