



## HSV 2 IgM EIA kit

(For in vitro diagnostic use only)

Enzyme-linked immunosorbent assay for the detection of IgM antibody to Herpes Simplex Virus 2 (HSV 2) infection

### INTENDED USE

The HSV 2 IgM kit is intended for use in the detection of IgM antibodies to Herpes Simplex Virus, type 2 (HSV 2) infection.

### SUMMARY AND PRINCIPLE OF THE TEST

Herpes Simplex Virus is a common pathogen and its primary infection is usually asymptomatic. There are two immunologically distinct types of HSV: Type 1 and Type 2. HSV 1 is generally associated with oral infection and lesions above the waist, and HSV 2 is associated with genital infections and lesions below the waist. Clinical cases primarily are 1) eczema herpeticum with eczematous skin changes with numerous lesions, 2) Gingivo-stomatitis and 3) Herpes sepsis, almost only found in newly born of premature infants. ATLAS ELISA HSV 1+ 2 IgM is an accurate serologic method to detect HSV 1 and 2 specific antibody IgM in serum sample.

ATLAS HSV 2 IgM capture kit utilizes ELISA based on the antibody-capture technique. Patient sera are incubated with mouse monoclonal antibody against human IgM bound to the solid surface of a microtiter well. Patient IgM is 'captured' by the surface bound antibody. Unbound serum components are washed away. Patient anti-HSV 2 IgM antibodies are 'detected' and bound by an immunocomplex, Enzyme conjugate, consisting of HSV 2 antigen which is conjugated to horseradish peroxidase. Unbound conjugate is removed by aspiration and washing. Substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The absorbance of this end product can be read spectrophotometrically at 450 nm and is directly proportional to the concentration of IgM antibodies to HSV 2 antigen present in the sample.

### REAGENTS

*Materials provided with the kits:*

1. 8X 12 well microtiter strip: 1 plate, coated with natural inactivated anti-human IgM.
2. Negative Control: 1 vial, 0.2ml
3. Positive Control: 1 vial, 0.2ml
4. Enzyme Conjugate: HRP-conjugated-HSV 2 antigen 6 ml
5. Wash Buffer: PBS, Tween. The buffer should be diluted with distilled water 1:20 before use. 40 ml
6. Substrate Solution A: urea peroxide. 6 ml
7. Substrate Solution B: TMB. 6 ml
8. Stop Solution: 2N Sulfuric Acid 6 ml

*Materials required but not provided.*

1. Micropipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled or deionized water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.

6. Microtiter plate or strip-well washer
7. Microtiter plate reader with 450nm wavelength
8. Timer

### PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Do not use kit beyond expiration date.
3. Do not mix components from kits with different lot number.
4. Avoid microbial contamination of reagents.
5. Do not pipette reagent by mouth and no smoking or eating while performing assays.
6. Wear gloves during the whole process and avoid reagents or specimen spilling-out.
7. Wipe up the spills using 5% hypochlorite solution.
8. Decontaminate all liquids or solid wastes before depositing.

### SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

### STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C. **DO NOT FREEZE KIT COMPONENTS.** The microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

### ASSAY PROCEDURE

1. Allow all components to reach room temperature before use.
2. Dispense 50 µl of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control, and 50µl of serum or plasma samples into respective test wells
3. Place the microtiter plate into a humidified box and incubate at 37C for 30 min.
4. Add 50 µl of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add Enzyme Conjugate to the blank well.
5. Place the microtiter plate into a humidified box and incubate at 37C for 30 min.
6. Wash each well 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
7. Add 50 µl of Substrate Solution A (HRP substrate) to each well, and then add 50 µl of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 min.
8. Add one drop (50 µl) of Stop Solution to each well to stop the color reaction. Read OD values of all samples at 450 nm.

### INTERPRETATION OF RESULTS

**EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):**

Cut Off: 0.10 + Average OD value of Negative Control

Positive: OD value is equal to or greater than the Cut Off value

Negative: OD value is less than the Cut Off value

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

#### LIMITATIONS OF THE ASSAY

1. Only if test instructions are rigidly followed will optimum results be achieved.
2. Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as washing the test strips and thorough mixing of all prepared solutions.
3. Results from neonate testing must be interpreted with care. Serum from the neonate should be tested in parallel with mother's serum. IgM antibody present in the neonate's serum is indicative of congenital infection if there has been no placental leakage. If the neonate has congenital infection, the IgM antibody may persist or rise. If the antibody is maternal, the IgM antibody level will drop.
4. Some antinuclear antibodies have been found to cause a false positive reaction on some ELISA tests.
5. A positive test does not guarantee protection from the disease. Any laboratory test result should be interpreted by the physician according to clinical findings.
6. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
7. A negative serological test does not exclude the possibility of past infection. Following primary HSV infection, antibody may fall to undetectable levels and then be boosted by later clinical infection with the same or heterologous type. Such a phenomenon may lead to incorrect interpretations of seroconversion and primary infection, or negative antibody status. In addition, samples obtained too early during primary infection may not contain detectable antibody. Some persons may fail to develop detectable antibody after Herpes infection.

#### REFERENCES

1. Oxman, M.N., D.D. Richman & S.A. Spector. 1982. Management at delivery of mother and infant when herpes simplex, varicella zoster, hepatitis, or tuberculosis have occurred during pregnancy. In: *Current Clinical Topics in Infectious Diseases*: pp 224-280
2. Nahmias, A.J., & W.E. Josey. 1982. Herpes simplex viruses 1 and 2. In: *Viral Infections of Humans*, Evans, A.S. ed. 2 nd Edition. New York: Plenum Press: p 351
3. Genital herpes infection - United States, 1966-1979. 1982. *Morbid Mortal. Wkly Rep.* 31:11.
4. Gardner, H.I. 1979. Herpes genitalis: Our most important venereal disease. *Am. J. Obstet. Gynecol.* 135:553
5. Bolognese, R.J., et al. 1976. Herpesvirus hominis type II infections in asymptomatic pregnant women. *Obstet. Gynecol.* 48:507
6. Sumaya, C.V., et al. 1980. Genital infections with herpes simplex virus in a university student population. *Sex Trans. Dis.* 7:16
7. Stewart, J.A., & K.L. Herrmann. 1986. Herpes Simplex Virus. In: *Manual of Clinical Laboratory Immunology*. Rose, N.R., Friedman, H., Fahey, J.L., eds. 3rd Edition. Wash, D.C. ASM. pp 497-501.
8. Rawls, W.E., & J. Campione-Piccardo. 1981. Epidemiology of herpes simplex virus type 1 and 2 infections. In: *The human herpesviruses: an interdisciplinary perspective*. Nahmias, A.J., Dowdle, W.R., and Schinazi, R.F., eds. New York: Elsevier: pp 137-152.

ATLAS MEDICAL  
William James House, Cowley Rd,  
Cambridge, CB4 4WX, UK  
Tel: ++44 (0) 1223 858 910  
Fax: ++44 (0) 1223 858 524

PPI374A01  
Revision A (13.08.2006)