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ELISA **E**NZYME **L**INKED **I**MMUNO**S**ORBENT **A**SSAY

Microwell Method

HSV 1+2 IgG

REF: V00025

For in vitro Diagnostic Use

P r o d u c t I n s e r t

Enzyme Linked Immunosorbent Assay for the **qualitative** determination of IgG Antibodies to Herpes Simplex Virus (HSV) type 1 and 2 in human serum or plasma. It is intended for screening and as an aid in the diagnosis of possible HSV 1+2 infection.

CE IVD

Microwell Method - 96 wells

(12 x 8-well Antigen coated Strips

Individual breakaway)

INTRODUCTION

Herpes Simplex Virus (HSV) is an envelope DNA virus belonging to the Herpes virus family which has been characterized into two distinct serotypes, HSV 1 and HSV 2. Infection with HSV 1 typically causes oral infections, whereas HSV 2 typically affects genital or neonate infection.

Primary HSV 1 infections usually occur in early childhood causing no symptoms. If symptoms are present, it can cause serious infection of gums, mouth, tongue, face and/or pharynx. Reactivation of the virus can lead to fever blisters or cold sores as well as ocular herpes. A majority of primary HSV 2 infection occurs mostly through sexual contact, with rare occasions occurring before onset of sexual activity. HSV 2 is typically asymptomatic but may present itself as genital herpes, characterized by bilaterally distributed lesions in the genital area accompanied by fever, inguinal lymphadenopathy and dysuria. Primary genital HSV is mainly caused by HSV 2, however approximately 15% can be attributed to HSV 1. Since HSV 1 unlikely produces recurrent infections, 99% of recurrent genital herpes is caused by HSV 2.[1]

One of the most serious consequences of genital herpes is neonatal herpes.[1] For newborns, almost all HSV 2 infections are acquired during birth through an infected birth canal.[2] Without therapy, untreated infants have more than 70% mortality rate with half survivors developing neurological impairment.[1,3] The presence of IgG antibodies to HSV is indicative of previous infection while a significant increase is indicative of reactivation, current or recent infection.

Primary infection is determined by presence of IgM antibodies.

The HSV 1+2 IgG ELISA Test Kit is an immunoassay for the qualitative detection of the presence of IgG antibodies to HSV in serum or plasma specimen.

PRINCIPLE OF THE ASSAY

The HSV 1/2 IgG ELISA Test Kit is a solid phase enzyme immunoassay based on indirect principle for the qualitative detection of IgG antibodies to HSV in human serum or plasma. The microwell plate is coated with HSV 1 and HSV 2 recombinant antigens. During testing, the specimen diluent and specimens are added to the antigen coated microwell plate and then incubated. If the specimens contain antibodies to HSV 1 or HSV 2, it will bind to the antigens coated on the microwell plate to form immobilized antigen-HSV 1 antibody complexes. If the specimens do not contain antibodies to HSV 1, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme-conjugated anti-human IgG antibodies are added to the microwell plate and then incubated. The enzyme-conjugated antihuman IgG antibodies will bind to the immobilized antigen-HSV antibody complexes present. After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of HSV IgG antibodies present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of HSV IgG antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

MATERIALS PROVIDED

1. **Microwell plate**: 12x 8-wells strips coated with recombinant HSV 1 antigen and recombinant HSV 2 antigen
2. **Enzyme Conjugate**: 1 vial of 12 mL; Anti-human IgG antibody bound to peroxidase; Preservative: 0.1 % ProClin™ 300
3. **Wash Buffer conc.**: 1 vial of 50 mL; 25x conc., Tris-HCl buffer containing 0.1 % Tween 20; Preservative: 0.1 % ProClin™ 300
4. **Specimen Diluent**: 1 vial of 12 mL; Tris buffer, Preservative: 0.1 % ProClin™ 300
5. **Substrate A**: 1 vial of 8 mL; Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1 % ProClin™ 300
6. **Substrate B**: 1 vial of 8 mL; Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1 % ProClin™ 300
7. **Stop Solution**: 1 vial of 8 mL; 0.5 M Sulfuric acid
8. **HSV 1+2 IgG Negative Control**: 1 vial of 1 mL; Diluted human serum containing no HSV 1+2 IgG antibodies; Preservative: 0.1 % ProClin™ 300
9. **HSV 1+2 IgG Cut-Off Calibrator**: 1 vial of 1 mL; Diluted human serum containing low titer IgG antibody against HSV; Preservative: 0.1 % ProClin™ 300
10. **HSV 1+2 IgG Positive Control**: 1 vial of 1 mL; Diluted human serum containing high titer IgG antibody against HSV; Preservative: 0.1 % ProClin™ 300
9. **Plate sealer**
10. **Package Insert**

MATERIALS REQUIRED BUT NOT PROVIDED

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 37°C ± 2°C
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Calibrated micropipettes with disposable tips capable of dispensing 5, 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Timer
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter
- Automated processor (optional)

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.

- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.
- Positive Control, Negative Control, Cut-Off Calibrator, Enzyme Conjugate, Sample Diluent, Substrate Solution A, Substrate Solution B, Wash Buffer:
  Above reagents contain 0.1 % ProClin™ 300 as a preservative, which is classified as below:

Warning

H317:	May cause an allergic skin reaction.
P272:	Contaminated work clothing should not be allowed out of the workplace.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352:	If on skin: wash with plenty of soap and water.
P333+P313:	If skin irritation or rash occurs: Get medical advice/attention.
P362+P364:	Take off contaminated clothing and wash it before reuse.
P501:	Dispose of contents and container in accordance to local, regional, national and international regulations.

HEALTH AND SAFETY INFORMATION

- Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Specimen Diluent, Substrate and Calibrators. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not mouth pipette.
- Avoid any contact of the Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution contains 0,5M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of

human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

STORAGE AND STABILITY OF THE KIT

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and removing the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch at 2-8°C and can be used within 3 months of the opening date.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The HSV 1+2 IgG ELISA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimes with fibrin particles or contaminated with microbial growth.
- Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS PREPARATION

WASH BUFFER:

Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle in a graduated cylinder and fill it with freshly distilled or deionized water to 1250 mL. It is stable for 2 weeks at 15-30°C.

Note: If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve.

ASSAY PROCEDURE

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the calibrators so that well A1 is the Blank well. From well A1, arrange the calibrators in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

1. Leave A1 as Blank well.
2. Add 100 µL of Negative Control in wells B1 and C1. (Blue Reagent)
   Add 100 µL of Cut-Off Calibrator in wells D1 and E1. (Blue Reagent)
   Add 100 µL of Positive Control in wells F1 and G1. (Red Reagent)
3. Add 100 µL of Specimen Diluent to assigned wells starting at H1. The color of Specimen Diluent is green.
   Add 5 µL of specimen to assigned wells starting at H1. Then a color change from green to blue will occur to verify that the specimen has been added.
   Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.
4. Mix gently by swirling the microwell plate on a flat bench for 30 seconds.
   Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.
5. Remove the Plate Sealer.
   Wash each well 5 times with 350 µL of Working Wash Buffer per well, then remove the liquid.
   Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried.
   Note: Improper washing may cause false positive results.
6. Add 100 µL of Conjugate to each well except for the Blank well. The color of Conjugate is red.
7. Cover the microplate plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.
8. Repeat step 5.
9. Add 50 µL of Substrate A to each well. (Clear Reagent)
   Add 50 µL of Substrate B to each well. (Clear Reagent)
   Then a blue color should develop in wells containing Positive specimens.
10. Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator at 37°C ± 2°C for 10 minutes ± 1 minute.
11. Remove the Plate Sealer.
    Add 50 µL of Stop Solution to each well. (Clear Reagent)
    Then a yellow color should develop in wells containing Positive specimens.
12. Read at 450/630-700 nm within 30 minutes.
    Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results

ASSAY SCHEME

1. Prepare the Working Wash Buffer by diluting the Wash Buffer concentrate 1:25.
2. Follow this scheme:

REAGENTS	A1 BLANK	CONTROLS	SAMPLE
Calibrators	-	100 µL	-

Sample Diluent	-	-	100 µL
Sample	-	-	5 µL
Cover strips with adhesive film.			
Incubate 30 min. at +37°C.			
Peel out the adhesive film and aspirate the reaction solution from all wells.			
Wash 5 times with 350 µL of diluted Wash Buffer, carefully aspirating off the remaining liquid.			
Enzyme Conjugate	-	100 µL	100 µL
Cover strips with adhesive film.			
Incubate 30 min. at +37°C.			
Peel out the adhesive film and aspirate the reaction solution from all wells.			
Wash 5 times with 350 µL of diluted Wash Buffer, carefully aspirating off the remaining liquid.			
Substrate A	50 µL	50 µL	50 µL
Substrate B	50 µL	50 µL	50 µL
Cover strips with a new adhesive film.			
Incubate 10 min. at +37°C., protected from light.			
Stop Solution	50 µL	50 µL	50 µL
Read the absorbance of each well against A1 blanking-well at 450 nm and 630-700 nm in 30 min.			

AUTOMATED PROCESSING

Automatic ELISA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic ELISA microplate processors are used, periodic validation is recommended to ensure proper results.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

1. Calculate the Mean Absorbance of Negative Control, Cut-Off Calibrator, and Positive Control by referring to the table below.

Example of Cut-Off Calibrator Calculation

Item	Absorbance
Cut-Off Calibrator: Well D1	0.328
Cut-Off Calibrator: Well E1	0.354
Total Absorbance of Cut-Off Calibrator	0.328 + 0.354 = 0.682
Mean Absorbance of Cut-Off Calibrator	0.682/2 = 0.341

2. Check the validation requirements below to determine if the test results are valid.

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm Note: It should be < 0.100 if read at 450 nm
Negative Control	Mean Absorbance after subtraction of Blank Absorbance should be < 0.100
Cut-Off Calibrator	Mean Absorbance after subtraction of Blank Absorbance should be > 0.150
Positive Control	Mean Absorbance after subtraction of Blank Absorbance should be > 1.500

NOTE: The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

INTERPRETATION OF RESULTS

Qualitative

Calculate the Index Value to obtain qualitative specimen results.

1. If the test is valid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean Absorbance of Cut-Off Calibrator. See an example of Cut-Off calculation below.

Item	Absorbance
Blank Absorbance: Well A1	0.001
Cut-Off Value: Mean Absorbance of Cut-Off Calibrator – Blank Absorbance	0.341 – 0.001 = 0.340

2. Calculate the Index Value by dividing the Specimen Absorbance by the Cut-Off Value, then read the results by referring to the Interpretation of Results table below.

Item	Absorbance
Specimen: Well H1	2.336
Cut-Off Value	0.340
Index Value: Specimen/Cut-Off Value	2.336/0.340 = 6.871

Interpretation of Results – Qualitative

Results	Qualitative
	Index Value
Negative	< 0.9
Positive	> 1.1
Equivocal*	≥ 0.9 and ≤ 1.1

*NOTE: For Equivocal results, the specimen should be retested. Specimens that are repeatedly Equivocal after retest should be confirmed using an alternate method. If the results remain Equivocal, collect a new specimen in two weeks. If the new specimen is Positive, the specimen is presumed to be Positive.

LIMITATIONS

1. The HSV 1+2 IgG ELISA Test Kit is used for the detection of IgG antibodies to HSV 1 and 2 in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test results. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A negative test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. As with other sensitive immunoassays, there is the possibility that the positive result cannot be repeated due to inadequate washing from initial testing. The results may be affected due to procedural or instrument error.
4. The Positive Control in the test kit is not to be used to quantify assay sensitivity. The Positive Control is used to verify that the test kit components are capable of detecting a Positive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The HSV 1+2 IgG ELISA Test Kit has correctly identified specimens of a mixed titer performance panel and has been compared to a leading commercial HSV 1 IgG ELISA test and HSV 2 IgG ELISA test using clinical specimens. The results show that the clinical sensitivity of the HSV 1+2 IgG ELISA Test Kit is 98.6%, and the clinical specificity is 98.0%.

HSV 1+2 IgG ELISA vs. Other ELISA

Method		Other ELISA		Total Results
HSV 1+2 IgG ELISA	Results	Positive	Negative	
	Positive	146	1	147
	Negative	2	49	51
Total Results		148	50	198

Clinical Sensitivity: 98.6% (95.2%-99.8%)*
Clinical Specificity: 98.0% (89.4%-99.9%)*
Overall Agreement: 98.5% (95.6-99.7%)*
*95% Confidence Interval

REPRODUCIBILITY

Intra-Assay: Within-run precision has been determined by using 10 replicates of three specimens: two low positive and a medium positive

Inter-Assay: Between-run precision has been determined by 3 independent assays on the same three specimens: two low positive and a medium positive. Three different lots of the HSV 1+2 IgG ELISA Test Kit have been tested using these specimens over a 5-day period.

Specimen	Intra-Assay			Inter-Assay		
	Mean Absorbance / Cut-Off	Standard Deviation	Coefficient of Variation (%)	Mean Absorbance / Cut-Off	Standard Deviation	Coefficient of Variation (%)
1	1.782	0.127	7.127	1.867	0.155	8.302
2	2.031	0.134	6.598	2.116	0.157	7.420
3	4.229	0.368	8.702	4.413	0.352	7.976

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