Foresight®

HSV 1 IgM EIA Test Kit
Package Insert

| REF I231-1161 | English |

An enzyme immunoassay (EIA) for the qualitative detection of IgM antibodies to Herpes Simplex Virus (HSV) type 1 in human serum or plasma.
For professional in vitro diagnostic use only.

INTENDED USE

The HSV 1 IgM EIA Test Kit is an enzyme immunoassay for the qualitative detection of IgM antibodies to HSV 1 in human serum or plasma. It is intended for screening and as an aid in the diagnosis of possible HSV 1 infection.

SUMMARY

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, 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 IgM EIA Test Kit is an immunoassay for the qualitative detection of the presence of IgM antibodies to HSV 1 in serum or plasma specimen. The test utilizes recombinant HSV 1 antigens to selectively detect IgM antibodies to HSV 1 in serum or plasma.

PRINCIPLE

The HSV 1 IgM EIA Test Kit is a solid phase enzyme immunoassay based on immunocapture principle for the qualitative detection of IgM antibodies to HSV 1 in human serum or plasma. The microwell plate is coated with anti- human IgM antibodies. During testing, the specimen diluent and specimens are added to the antibody coated microwell plate and then incubated. If the specimens contain IgM antibodies to HSV 1, it will bind to the antibodies coated on the microwell plate to form immobilized anti-human IgM antibody-HSV 1 IgM antibody complexes. If the specimens do not contain IgM 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 recombinant HSV 1 antigens are added to the microwell plate and then incubated. The enzyme-conjugated recombinant HSV 1 antigens will bind to the immobilized anti-human IgM antibody-HSV 1 IgM 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 1 IgM 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 1 IgM antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

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.

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, Calibrators and Controls. 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 pipette by mouth.
- 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

- 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 remove the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch with desiccant supplied at 2-8°C and can be used within 3 months of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seal closure to seal the pouch completely and immediately store at 2-8°C.
- 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 to the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The HSV 1 IgM EIA 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 specimens with fibrin particles or contaminated with microbial growth.
- 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 AND COMPONENTS

Materials Provided

No.	Reagent	Component Description	Quantity		
			96 wells/kit	480 wells/kit	48 wells/kit
	HSV 1 IgM Microwell Plate	Microwell plate coated with anti-human IgM antibodies	1 plate (96 wells/plate)	5 plates (96 wells/plate)	1 plate (48 wells/plate)
1	HSV 1 IgM Conjugate	Recombinant HSV 1 antigens bound to peroxidase; Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL	1 x 6 mL
2	Concentrated Wash Buffer (25x)	Tris-HCl buffer containing 0.1% Tween 20; Preservative: 0.1% ProClin™ 300	1 x 50 mL	5 x 50 mL	1 x 25 mL
2A	Specimen Diluent	Tris buffer; Preservative: 0.1% ProClin™ 300	1 x 12 mL	5 x 12 mL	1 x 6 mL
3	Substrate A	Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL	1 x 4 mL
4	Substrate B	Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL	1 x 4 mL
5	Stop Solution	0.5M Sulfuric acid	1 x 8 mL	5 x 8 mL	1 x 4 mL
6	HSV 1 IgM Negative Control	Diluted human serum non-reactive for HSV 1 IgM antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
7	HSV 1 IgM Cut-Off Calibrator	Diluted human weakly reactive for HSV 1 IgM antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
8	HSV 1 IgM Positive Control	Diluted human highly reactive for HSV 1 IgM antibodies; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL	1 x 0.5 mL
	Plate Sealers		3	15	3
	Package Insert		1	1	1

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)

DIRECTIONS FOR USE

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 controls so that well A1 is the Blank well. From well A1, arrange the controls in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

Step	Detailed Procedure	Simplified Procedure
	- Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle containing the concentrated wash buffer in a graduated cylinder and fill it with freshly distilled or deionized water to 1250 mL for 96 wells/plate testing, or 625 mL for 48 wells/plate testing. The Working Wash Buffer 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. - Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.	- Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 - Remove and store unused strips at 2-8°C
0	- Leave A1 as Blank well.	- Leave A1 as Blank well
1	- 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)	- B1 and C1: Add 100 µL Negative Control - D1 and E1: Add 100 µL Cut-Off Calibrator - F1 and G1: Add 100 µL Positive Control
2	- Add 100 µL of Specimen Diluent to assigned wells starting at H1. (Green Reagent) - 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.	- Starting H1: Add 100 µL Specimen Diluent - Starting H1: Add 5 µL specimen

3	• 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.	• Mix gently • Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
4	• 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.	• Remove the Plate Sealer • Wash each well 5 times with 350 µL of Working Wash Buffer • Turn the microwell plate upside down on absorbent tissue
5	• Add 100 µL of Conjugate to each well except for the Blank well. (Red Reagent)	• Add 100 µL of Conjugate to each well except for the Blank well
6	• 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.	• Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
7	• Repeat Step 4.	• Repeat Step 4
8	• 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.	• Add 50 µL of Substrate A to each well • Add 50 µL of Substrate B to each well
9	• 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.	• Mix then cover microwell plate with Plate Sealer and incubate at 37°C for 10 min
10	• 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.	• Remove Plate Sealer • Add 50 µL of Stop Solution to each well
11	• 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.	• Read at 450/630-700 nm within 30 min

AUTOMATED PROCESSING

Automatic EIA 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 EIA 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	0.305
Cut-Off Calibrator: Well E1	0.317
Total Absorbance of Cut-Off Calibrator	0.305 + 0.317 = 0.622
Mean Absorbance of Cut-Off Calibrator	0.622/2 = 0.311

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 > 0.800

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	0.004
Cut-Off Value: Mean Absorbance of Cut-Off Calibrator – Blank Absorbance	0.311- 0.004 = 0.307

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	0.751
Cut-Off Value	0.307
Index Value: Specimen/Cut-Off Value	0.751/0.307= 2.446

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 IgM EIA Test Kit is used for the detection of IgM antibodies to HSV 1 in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. 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 the initial test. 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 IgM EIA Test Kit has correctly identified specimens of a mixed titer performance panel and has been compared to a leading commercial HSV 1 EIA test using clinical specimens. The results show that the clinical sensitivity of the HSV 1 IgM EIA Test Kit is 96.3%, and the clinical specificity is 99.5%.

HSV 1 IgM EIA vs. Other EIA

Method		Other EIA		Total Results
	Results	Positive	Negative	
HSV 1 IgM EIA	**Positive**	77	3	80
	Negative	3	609	612
Total Results		80	612	692

Clinical Sensitivity: 96.3% (89.4-99.2%)*   Clinical Specificity: 99.5% (98.6-99.9%)*
Overall Agreement: 99.1% (98.1-99.7%)*   *95% Confidence Interval

Reproducibility

Intra-Assay: Within-run precision has been determined by using 10 replicates of two specimens: a low positive and a medium positive.
Inter-Assay: Between-run precision has been determined by 3 independent assays on the same two specimens: a low positive and a medium positive. Three different lots of the HSV 1 IgM EIA Test Kit have been tested using these specimens over a 5-day period.
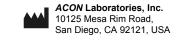
Specimen	Intra-Assay			Inter-Assay		
	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)
1	0.524	0.023	4.389	0.552	0.039	7.065
2	0.781	0.044	5.633	0.811	0.042	5.179

BIBLIOGRAPHY

1. Arvin, C, Prober. Herpes Simplex Viruses. In: Manual of Clinical Microbiology 6th Ed. (1995) 876-883.
2. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines. 2002. MMWR RR-6 2002:51.
3. Whitley, R. Herpes Simplex Viruses. In: Fields Virology 3rd Ed. (1996) 2297-2231.

Index of Symbols

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
[i]	Consult instructions for use	Σ	Tests per kit	(Manufacturer symbol)	Manufacturer
IVD	For *in vitro* diagnostic use only	(hourglass)	Use by	EC REP	Authorized Representative
		LOT	Lot Number	(thermometer)	Store between 2-8°C
HSV 1 IgM	HSV 1 IgM	Substrate A	Substrate A	Substrate B	Substrate B
Wash Buffer 25x	Wash Buffer (25x)	Conjugate	Conjugate	Control +	Positive Control
Calibrator Cut-Off	Cut-Off Calibrator	Control −	Negative Control	REF	Catalog #
Microwell Plate	Microwell Plate	Plate Sealer	Plate Sealer		
Specimen Diluent	Specimen Diluent	Stop Solution	Stop Solution	Package Insert	Package Insert