11. Assay Procedure Continued…

8. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate **(Reagent 4)** into each well. Incubate the plate for **10 minutes**.
9. Add 100µl of Stop Solution **(Reagent 5)** to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.
10. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

12. Quality Control

Quality control data is supplied on the lot-specific QC certificate included in the kit. Controls are intended to monitor for substantial reagent failure. Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD-values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

13. Interpretation of Results

Semi-Quantitative Results

Plot the optical densities of the 0, 25 and 100 U/ml standards and the positive control (150 U/ml) against concentration and join the points. Read the concentrations of the unknowns from this graph. Concentrations above 10 U/ml are considered positive.

Qualitative Results

Negative samples: OD < OD of 10 U/ml standard
Positive samples: OD >/= OD of 10 U/ml standard

1. HSV-1 and HSV-2 share many cross-reacting antigens. Therefore, to fully evaluate the IgG antibody status to HSV, both the HSV-1 and HSV-2 ELISA tests should be run simultaneously on each sample. The results of both tests should be compared and evaluated as follows:

	Interpretation
Positive HSV -1 / HSV-2	Pos for IgG antibody to HSV indicates a current or previous infection with HSV-1, HSV-2 or both
Negative HSV-1 / HSV-2	Negative for IgG antibody to HSV indicates no current or previous infection with HSV-1 or HSV-2

2. Specimens taken too early during a primary infection may not have detectable levels of IgG antibody. If a primary infection is suspected, another specimen should be taken in 8-14 days and tested concurrently in the same assay with original specimen to look for seroconversion.
3. To evaluate acute and convalescent sera, both samples must be tested in the same assay. If the acute specimen is negative and the convalescent specimen is positive for IgG antibody to either HSV-1 or HSV-2 or both, seroconversion has taken place and a primary HSV infection is indicated.

14. Limitations of the Procedure

1. Initial infection with HSV-2 in persons with a past infection with HSV-1 will likely produce a significant rise in antibody titre to common antigens as well as to HSV-2.
2. HSV-1 or HSV-2 antibody test results will not indicate the site of infection. The test is not intended to replace viral isolation.
3. The presence of IgG antibodies to HSV-1 or HSV-2 does not necessarily assure protection from future infection with HSV-1.
4. The antibody titre of a single serum specimen cannot be used to determine recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to demonstrate seroconversion.
5. Test results for demonstration of seroconversion should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
6. A positive HSV IgG test in neonates should be interpreted with caution since passively acquired maternal antibody can persist for up to 6 months. However, a negative test for IgG antibody in the neonate may help exclude congenital infection. The most definitive diagnosis of active HSV infection requires viral isolation.
7. Specimens containing antibodies to nuclear antigens may give false positive results.
8. The incidence of HSV infection varies with age, geographical location, sexual behaviour and socio-economic status.

15. Performance Characteristics

The Genesis Diagnostics HSV-2 IgG kits were compared with other commercially available ELISA assays for the detection of IgG antibodies to HSV-2. The results are summarised below.

n=80	Reference +	Reference -	Reference +/-
Genesis +	33	2	7
Genesis -	0	38	0

16. Reproducibility

Within Assay Precision

CV%: <12%

Between Assay Precision

CV%: <12%

17. Method Summary

- Dilute sera 1:100 with sample diluent **(Reagent 1)**
- Dispense 100µl of each control, the standards and diluted sample into the microplate wells
- Incubate for **20 minutes** at room temperature.
- *Wash the wells three times*
- Dispense 100µl of Conjugate **(Reagent 3)** into each well
- Incubate at room temperature for **20 minutes**
- *Wash the wells four times*
- Add 100µl of TMB substrate **(Reagent 4)** to each well
- Incubate at room temperature for **10 minutes**
- Add 100µl Stop solution **(Reagent 5)** to each well
- Read the optical density at 450nm

18. Further Reading

Denoyei Ga, *et al*: Enzyme immunoassay for measurement of antibodies to Herpes Simplex Virus infection: Comparison of complement fixation, immunofluorescent antibody and neutralisation techniques. J. Clin. Micro. 11: 114-119, 1980
Cremer NE *et al*: Evaluation and reporting of enzyme immunoassay determinations of antibody to Herpes simplex Virus in sera and cerebrospinal fluid. J. Clin. Micro. 15: 815, 1982
Nahmias AJ, and Roizman BR: Infection with Herpes Simplex Viruses 1 and 2. New Eng. J. Med. 289:667-781, 1983

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G·E·N·E·S·I·S Diagnostics

Herpes-2 IgG ELISA Kit

Semi-quantitative/qualitative assays for anti-Herpes simplex-2 IgG antibodies

Product Code: GD087

For *in vitro* Diagnostic Use

CE

1. Materials Included in the Kit

- **Microplate** 96 wells in 12 x 8 break-apart strips, pre-coated with inactivated HSV-2 strain antigen
- **Reagent 1**: **Sample Diluent** 150mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 10ml, (blue), **concentrate** (x15)
- **Reagent 2: Wash Buffer** 100mM Tris-buffered saline with detergent, pH 7.2, 100 ml, **concentrate** (x10)
- **Reagent 3: Conjugate** rabbit anti-human IgG (red) conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12ml, ready to use
- **Reagent 4: TMB Substrate** aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- **Reagent 5: Stop Solution** 0.25M sulphuric acid, 12 ml, ready to use
- **Standards:** 10 U/ml (for qualitative assays, yellow), 25 & 100 U/ml, 1ml of 10mM Tris-buffered saline with human serum IgG antibodies Herpes-2, ready to use
- **Positive control:** 1ml of 10mM Tris-buffered saline containing human serum antibodies to Herpes-2, (red), ready to use.
- **Negative control:** 1ml of 10mM Tris-buffered saline containing normal human serum, (green), ready to use.
- **Instructions for use**

263-087-04

2. Other Equipment Required

Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 5µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable, self-validated automated system may be used.

Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

3. Intended Use

The Herpes-2 IgG kit is an ELISA designed for the qualitative determination of IgG antibodies to Herpes Simplex Virus (HSV) in human serum/plasma. The assay is intended to be used to evaluate serologic evidence of primary or reactivated infection with HSV, and are for *in vitro* diagnostic use.

4. Explanation of the Test

Herpes simplex infections are caused by two antigenically distinct strains of the common virus Herpes simplex. HSV-1 is usually associated with infections in the oropharyngeal area and eyes while HSV-2 causes most genital and neonatal infections. Following infection, a latent infection is established in sensory neurons, and recurrent infection results from reactivation of latent infection. HSV infections are usually localised to the initial site of infection. However, serious localised or disseminated disease may occur in immunocompromised individuals including newborn infants, cancer patients and transplant recipients.

HSV infections are transmitted by virus-containing secretions through close personal contact. Infection is classified as either primary or recurrent. Both forms are often subclinical and asymptomatic. Primary symptomatic HSV-1 infections are characterised by gingivostomatitis associated with fever, malaise and tender swollen cervical lymph nodes. The most common form of recurrent HSV-1 is herpes labialis in which vesicles appear on the lips, nostrils or skin around the mouth. Genital HSV infections manifest as multiple ulcerative lesions accompanied by pain, fever, dysuria and lymphadenopathy.

The most severe complication of genital HSV infection is neonatal disease. HSV is transmitted from the mother to the neonate during birth. Of mothers with an active infection, the risk of transmission to infants is as high as 40%. About 69-80% of infants who develop neonatal herpes are born to women who are asymptomatic of genital herpes at the time of birth. Infants infected with HSV appear normal at birth but generally develop symptoms during the newborn period. Of the infants with neonatal HSV, about half will die if not treated, and about half of the surviving infants will develop severe neurological or ocular sequelae.

5. Principle of the Test

Diluted serum or plasma specimens (1:100) are incubated for 20 minutes to allow specific antibodies to HSV-2 to bind to the antigen-coated wells. After washing away unbound antibodies and other serum constituents, HSV specific IgG is detected using rabbit anti-human IgG conjugated to horseradish peroxidase. After 20 minutes incubation, unbound conjugate is removed by washing, and TMB enzyme substrate is added for 10 minutes. A blue colour develops if antibodies to HSV are present. Addition of stop solution gives a yellow colour and the absorbance of controls, standards and samples are measured using a microplate reader at 450nm or 450/620nm dual wavelength.

6. Safety Precautions

1. All reagents in this kit are for *in vitro* diagnostic use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. CAUTION: the device contains material of human and animal origin and should be handled as a potential transmitter of diseases. All human source material used in the preparation of standards and control for this product have been tested and found negative by ELISA for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Dispose of plates and specimens as clinical waste. Any unused reagents should be flushed away with copious amounts of water. Disposal must be performed in accordance with local legislation.

7. Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 – 8°C after use.
3. The sample diluent X15 concentrate contains 0.09% sodium azide as preservative. Prepare suffiicient working strength diluent for the assay run. However, if the working strength diluent is to be stored for more than 1 week, add sodium azide (0.9g/L). Store unused sample diluent concentrate and dilute sample diluent at 2 – 8°C.
4. Include the Positive and Negative Control in every test run to monitor for reagent stability and correct assay performance.
5. Strictly observe the indicated incubation times and temperature.
6. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
7. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent.
8. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
9. Do not allow microwells to dry between incubation steps.
10. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
11. Avoid direct sunlight and exposure to heat sources during all incubation steps.
12. Replace colour-coded caps on their correct vials to avoid cross-contamination
13. It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

8. Shelf Life and Storage Conditions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer and Sample Diluent (see Technical Precautions) have a shelf life of 3 months if stored in a closed bottle at 2 - 8°C.

9. Specimen Collection and Storage

Serum and plasma samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

10. Preparation of Reagents

1. Dilute the Sample Diluent **(Reagent 1)** 1:14 in distilled water to make sufficient buffer for the assay run e.g. add 10ml sample diluent concentrate to 140 ml water.
2. Dilute the Wash Buffer **(Reagent 2)** 1:9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water.

11. Assay Procedure

1. Dilute patient samples 1:100 (e.g. 5µl serum plus 0.5 ml diluent). It is important to dispense all samples, standards and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.
2. Assemble the number of strips required for the assay.
3. For qualitative assays, dispense 100 µl of the negative control, the 10 U/ml standard, the positive control and the diluted patient sample into the wells. For semi-quantitative assays, also dispense 100 µl of the sample diluent as the 0 U/ml standard.
4. Incubate for **20 minutes** at room temperature. During all incubations, avoid direct sunlight and close proximity to any heat sources.
5. After 20 minutes, decant or aspirate the well contents and wash the wells 3 times using an automatic plate washer or the manual wash procedure (see below). Careful washing is the key to good results. Blot the wells on absorbent paper before proceeding. **Do not allow the wells to dry out.**
   <u>Manual Wash Procedure</u>
   Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with wash buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.
6. Dispense 100µl of Conjugate **(Reagent 3)** into each well. Incubate the wells for **20 minutes** at room temperature.
7. After 20 minutes, discard the well contents and carefully wash the wells 4 times with Wash Buffer. Ensure that the wells are empty but do not allow to dry out.