

**Herpes-2 IgG ELISA Kit**  
**Semi-quantitative/qualitative assays for**  
**anti-Herpes simplex-2 IgG antibodies**  
 Product code GD87  
 96 tests

100108

**1. Intended use**

The Herpes-1 IgG and Herpes-2 IgG kits are ELISAs designed for the qualitative determination of IgG antibodies to Herpes Simplex Virus (HSV) in human serum. Plasma samples may also be used. The assays are intended to be used to evaluate serologic evidence of primary or reactivated infection with HSV, and are for *in vitro* diagnostic use. Due to cross-reactivity of shared antigens, both tests must be run in parallel on the same sample to fully evaluate a patient serum.

**2. Introduction**

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.

**3. 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.

**4. 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 (peroxidase conjugated rabbit anti-human IgG), 12 ml, (red). Ready to use
- **Reagent 4:** TMB substrate, 12 ml. Ready to use
- **Reagent 5:** Stop solution, 12 ml. Ready to use
- **Positive control:** (red), 150U/ml, 1ml liquid. Ready to use.
- **Standards:** 10 U/ml (for qualitative assays, yellow), 25 & 100 U/ml (blue), 1ml liquid. Ready to use.
- **Negative control:** (green), 1ml liquid. Ready to use.
- **Instructions for use**

**5. Other equipment required**

10mm X 60mm tubes for dilution, pipettes 5µl, 100µl, 1000µl; repeating dispenser 100µl, microplate reader with 450nm filter, microplate washing device. Distilled or de-ionised water, general laboratory apparatus.

**6. Storage and precautions**

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for three months (or until its expiry date if less than three months). It is important to protect the unused wells from excess moisture. Do not use kits beyond their expiry date.

The standards and controls are manufactured from dilute non-infectious human serum. Normal clinical laboratory safety procedures should be maintained at all times. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.

The stop solution contains 0.25M sulphuric acid.

**7. Samples**

Only freshly drawn and properly refrigerated sera or plasma should be used in this assay. Avoid hemolysed, lipemic or bacterial contaminated sera. Sera should be stored at 2-8°C for no longer than 5 days. If a delay in testing is anticipated, store test sera at -20°C. Avoid multiple freeze-thaw cycles.

**8. Method**

Ensure that all materials are at room temperature before beginning the procedure. We recommend that the standards and the controls are always run in duplicate. Samples may be run singly or in duplicate.

1. Assemble the number of strips required for the assay.
2. The sample diluent X15 concentrate contains 0.09% sodium azide as preservative. Prepare sufficient 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. 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.
3. 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.
4. 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 sample diluent as 0 U/ml and 100 µl of the 25 and 100 U/ml standards.
5. Incubate for **20** minutes at room temperature. During all incubations, avoid direct sunlight and close proximity to any heat sources.
6. 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. The diluted wash buffer is stable for two months at 2 - 8°C.
7. 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.**  
  
Manual Wash Procedure:  
 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.
8. Dispense 100µl of Conjugate (**Reagent 3**) into each well. This reagent is colour coded red. Keep all pipettes and other equipment used for Conjugate completely separate from the

substrate reagent! Incubate the wells for **20** minutes at room temperature.

9. After 20 minutes, discard the well contents and carefully wash the wells 4 times with wash buffer. Ensure that the wells are completely washed. Blot the microplate on absorbent paper to remove final drops of wash fluid. **Do not allow the wells to dry out.**
10. Using a repeating dispenser, rapidly dispense 100µl of TMB substrate (**Reagent 4**) into each well. Incubate the plate for **10** minutes.
11. 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.
12. Read the optical density in a microplate reader at 450nm or 450/620nm within 10 minutes.

### 9. Quality control

The expected optical density values for the negative and positive controls and the 10 U/ml standard are given on the certificate included in the kit.

### 10. Interpretation

#### Semi-quantitative assays

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 assays

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:

Positive	Negative	Interpretation
HSV-2 HSV-1		Positive for IgG antibody to HSV indicates a current or previous infection with HSV-1 or HSV-2 or both
HSV-2 HSV-1	HSV-1 HSV-2	
	HSV-1 HSV-2	Negative for IgG antibody to HSV indicates no current or previous infection with HSV-1 or HSV-2. (see 2 below)

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. A positive result indicates a current or previous infection with HSV.
4. 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.

### 11. Limitations

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.

### 12. Expected Values

The incidence of HSV infection varies with age, geographical location, sexual behaviour and socioeconomic status.

### 13. Performance characteristics

#### Comparative study:

The Genesis Diagnostics HSV-1 and HSV-2 IgG kits were compared with other commercially available ELISA assays for the detection of IgG antibodies to HSV-1 and HSV-2. The results are summarised below.

Comparative Study (n= 80)	Reference HSV-1 IgG ELISA kit	
	+	-
Genesis Diagnostics HSV-1 IgG kit	47	1
	1	31

Comparative Study (n=80)	Reference HSV-2 IgG ELISA kit		
	+	-	+/-
Genesis Diagnostics HSV-2 IgG kit	33	2	7
	0	38	0

### 14. Assay characteristics

Within Assay Imprecision < 12%  
Between Assay Imprecision < 12%

#### 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

#### 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