

Anti-HSV-2 (gG2) ELISA (IgG)

Test instruction

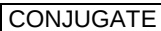
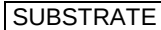
ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2532-9601-2 G	Herpes-simplex-virus type 2 (HSV-2), glycoprotein G2	IgG	Ag-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the immunoglobulin class IgG against the HSV-2 specific glycoprotein G2 in serum or plasma for the diagnosis of HSV-2 infections.

Application: HSV-2 glycoprotein G2 (gG2) is used as the antigen in this ELISA, allowing type-specific detection of IgG antibodies against HSV-2. A positive test result indicates contact with the virus. When acute processes are suspected, e.g. genital herpes, especially during pregnancy, or HSV encephalitis, direct detection should be performed.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with purified glycoprotein G2. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	
2. Calibration 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	
8. Sample buffer ready for use	light blue	1 x 100 ml	
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	
10. Chromogen/substrate solution TMB/H ₂ O ₂ , ready for use	colourless	1 x 12 ml	
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	
 Lot description			 Storage temperature
 In vitro diagnostic medical device			 Unopened usable until



Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.
- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.
- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.
- **Sample buffer:** Ready for use.
- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.
- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light . The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.
- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** sample buffer. For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The calibrators and controls are prediluted and ready for use, do not dilute them.



Incubation

For **semiquantitative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)

Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.
Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:

Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.

Automatic: Wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (>10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values.

Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.

Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)

Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.

Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:

Empty the wells. Wash as described above.

Substrate incubation: (3rd step)

Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C) (protect from direct sunlight).

Stopping:

Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:

Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.



Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on inquiry.

Automated test performance using other fully automated, open system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 2	P 6	P 14	P 22			C 1	P 4	P 12	P 20		
B	pos.	P 7	P 15	P 23			C 2	P 5	P 13	P 21		
C	neg.	P 8	P 16	P 24			C 3	P 6	P 14	P 22		
D	P 1	P 9	P 17				pos.	P 7	P 15	P 23		
E	P 2	P 10	P 18				neg.	P 8	P 16	P 24		
F	P 3	P 11	P 19				P 1	P 9	P 17			
G	P 4	P 12	P 20				P 2	P 10	P 18			
H	P 5	P 13	P 21				P 3	P 11	P 19			

The pipetting protocol for microtiter strips 1 to 4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).

The pipetting protocol for microtiter strips 7 to 10 is an example for the **quantitative analysis** of 24 patient sera (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.

The wells can be broken off individually from the strips. Therefore, the number of tests performed can be matched to the number of samples, minimising reagent wastage.

Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2. Calculate the ratio according to the following formula:

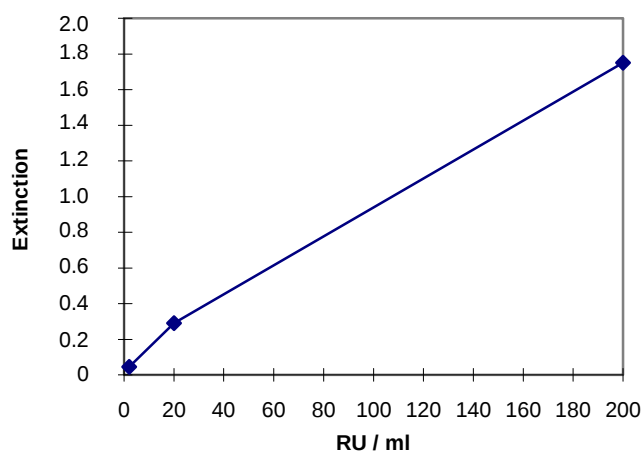
$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	negative
Ratio ≥0.8 to <1.1:	borderline
Ratio ≥1.1:	positive



Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use "point-to-point" plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction for a patient sample lies above the value of calibrator 1 (200 RU/ml), the result should be reported as ">200 RU/ml". It is recommended that the sample be retested at a dilution of e.g. 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range of non-infected persons (**cut-off value**) recommended by EUROIMMUN is **20 relative units (RU)/ml**. EUROIMMUN recommends interpreting results as follows:

<16 RU/ml:	negative
≥16 to <22 RU/ml:	borderline
≥22 RU/ml:	positive

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends retesting the samples.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. Significant titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after 7 to 10 days can indicate an acute infection. To investigate titer changes, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: As no international reference serum exists for antibodies against HSV-2, the calibration is performed in relative units (RU)/ml.

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratio values determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.



The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The microplate wells were coated with glycoprotein G2 purified by affinity chromatography. Glycoprotein G2 is an envelope protein which is only present in HSV-2 but not in HSV-1.

Linearity: The linearity of the Anti-HSV-2 (gG2) ELISA (IgG) was determined by assaying at least 4 serial dilutions of different patient samples. The coefficient of determination R^2 for all sera was > 0.95 . The Anti-HSV-2 (gG2) ELISA (IgG) is linear at least in the tested concentration range (3 RU/ml to 200 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit for the Anti-HSV-2 (gG2) ELISA (IgG) is 1.4 RU/ml.

Cross reactivity: This ELISA showed no cross reactivity. In particular, problems such as anti-HSV-1 antibodies that often cross react with anti-HSV-2 antibodies causing false positive results do not occur with this ELISA.

Antibodies against	n	Anti-HSV-2 (gG2) ELISA (IgG) positive
Adenovirus	12	0%
Chlamydia pneumoniae	12	0%
CMV	12	0%
EBV-CA	12	0%
Helicobacter pylori	12	0%
HSV-1	12	0%
Influenza virus A	12	0%
Influenza virus B	12	0%
Measles virus	12	0%
Mumps virus	12	0%
Mycoplasma pneumoniae	12	0%
Parainfluenza virus Pool	12	0%
RSV	12	0%
Rubella virus	12	0%
Toxoplasma	12	0%
VZV	12	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed on 6 different test runs.

Intra-assay variation, n = 20		
Sample	Mean value (RU/ml)	CV (%)
1	23	5.1
2	47	4.8
3	76	3.9

Inter-assay variation, n = 4 x 6		
Sample	Mean value (RU/ml)	CV (%)
1	25	7.3
2	49	4.2
3	77	3.2



Sensitivity and specificity: 168 clinically pre-characterised patient samples (INSTAND, Labquality and IQS) were investigated with the EUROIMMUN Anti-HSV-2 (gG2) ELISA (IgG). The sensitivity amounted to 100%, with a specificity of 100%. Borderline results were not included in the calculation

n = 168		INSTAND, Labquality, IQS		
		positive	borderline	negative
EUROIMMUN Anti-HSV-2 (gG2) ELISA (IgG)	positive	70	0	0
	borderline	0	1	1
	negative	0	3	93

Reference range: The levels of anti-HSV-2 antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of 20 RU/ml, 9.6% of the blood donors were anti-HSV-2 positive (IgG), which reflects the known percentage of infections in adults.

Clinical significance

The Anti-HSV-1/2 ELISA (IgG/IgM), Anti-HSV-1/2 EUROLINE-WB (IgG/IgM) and HSV IIFT as components of the TORCH Profile are used for the serological determination of antibodies against herpes simplex virus 1 (HSV-1, human-pathogenic herpes virus 1, HHV-1) and/or herpes simplex virus 2 (HSV-2, human-pathogenic herpes virus 2, HHV-2).

The two closely related human-pathogenic virus species HSV-1 and HSV-2 from the herpesviridae family are the causative agents of different diseases, which can be asymptomatic, benign or severe. Frequent diseases are herpes labialis and genitalis, while herpes simplex encephalitis and herpes neonatorum occur less often.

Herpes simplex is a disease which is prevalent worldwide and characterised by the formation of blisters on the skin and mucous membranes as non-intermittent, lytic replication in epithelial cells. Complications result when internal organs are also affected and necrotise.

HSV-1 infections generally affect the area of the mouth and nose. Infections with HSV-2 occur in the genital area. Individuals with promiscuous behaviour have a higher disease risk. With primary infection the virus enters the local mucosa cells and replicates in the epithelium. The herpes simplex virus then spreads within the epithelium by destroying the host cells and releasing new virions. As a result of the tissue destruction ulcers or inflammatory skin blisters develop. The liquid in these blisters is an exudate with a high virus concentration. The herpes simplex virus migrates to the nerve ends of sensitive neurons where it is taken up specifically and transported along the microtubules and intermediary filaments of the axons to the cell body of the nerve. The migration rate to the cell body is around 0.7 µm per second.

In primary HSV-2 infections, cephalalgia and neck stiffness are frequent symptoms, whereas meningitis is rarely observed. If newborns are infected with HSV-2 in the birth canal, blisters on the skin, mouth and eyes are observed, often accompanied by hepatomegaly and splenomegaly, renal failure, icterus, neurological damage and encephalitis. For this reason caesarean section is indicated in acute infections, regardless of primary infection or relapse. The case-fatality ratio in untreated disseminated HSV-2 infections is 60%. It is possible that the presence of anti-HSV-1 antibodies reduces the severity of HSV-2 infection.

The seroprevalence increases up to the age of 50. Provocable relapses are typical. However, the antibodies are not able to prevent relapses. Antibodies against HSV can be detected in the serum of nearly all patients after the disease has taken its course. For HSV-1 the known infection level in adults is 70% to 90%. Antibodies against HSV-2 can be found in only 7% to 20% of general population and in more than 20% of promiscuous adults. Mixed infection with both types is revealed in about 11%. Parallel infections of HIV-1 and HSV-2 have been observed in 95% of herpes simplex patients in Africa. HSV infections are the most frequent co-infections found in HIV-infected persons. HSV-2 micro and macro ulcerations in the genital mucosa can promote further infection.



Primary infections with HSV and relapses may lead to severe illness in pregnant women. The virus is transmitted transplacentally to the unborn child and can cause foetal infection. Infection of the unborn child can lead to intrauterine death, malformations and premature birth. Direct detection of the pathogen in infected tissue or lymph (CSF, ocular humour, blister liquid, etc.) has a high diagnostic value in HSV infections. It is generally performed by determining viral DNA using polymerase chain reaction (PCR) or, more rarely, by detection of virus-specific antigens using immunofluorescence. PCR is the method of choice for early detection of HSV infections during pregnancy, particularly to determine the necessity of caesarean section. For timely diagnosis of HSV encephalitis the determination of HSV DNA in CSF using PCR is indispensable. Virus isolation from cell cultures as a specific, direct detection method is also possible. This procedure, however, may take up several days due to the required differentiation of the produced viruses. Detection of the virus by electron-optical examination of infected material is possible, but the two species of the virus family herpesviridae cannot be differentiated based on their morphology.

In HSV meningitis/encephalitis agent-specific antibodies of class IgG are produced in CSF. The intrathecal agent-specific antibody production is defined by the relative CSF/serum quotient CSQ_{rel} . (synonym: antibody specificity index). The quotient is calculated from the amount of agent-specific antibodies in total CSF IgG in proportion to the amount of agent-specific antibodies in total serum IgG.

Type-specific serological diagnosis of herpes simplex virus infections requires test systems based on type-specific antigens, namely glycoprotein C1 (gC1) or glycoprotein G1 (gG1) of HSV-1 and glycoprotein G2 (gG2) of HSV-2. Due to the use of these highly purified proteins, which are isolated from the viral coats of herpes simplex virus species HSV-1 and HSV-2, both Westernblot and ELISA produce accurate results. The combination of a sensitive screening test (IIFT or ELISA) and a specific confirmation test (Westernblot) ensures reliable diagnosis of herpes simplex virus infections and exact differentiation between herpes simplex 1 and herpes simplex 2 infections.

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