

Anti-HSV-1 (gC1) ELISA (IgM)

Test instruction

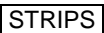



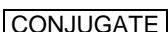









ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2531-9601-2 M	Herpes-simplex-1 virus (HSV-1, glycoprotein C1)	IgM	Ag-coated microplate wells	96 x 01 (96)

Indication: The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of the immunoglobulin class IgM against the HSV-1 specific glycoprotein C1 in serum or plasma for the diagnosis of herpes simplex 1 infections.

Application: HSV-1 glycoprotein C1 (gC1) is used as the antigen in this ELISA, allowing type-specific detection of IgM antibodies against HSV-1. 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 of the virus should be performed.

Principle of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with purified glycoprotein C1. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgM antibodies (also IgA and IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (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. Calibrator (IgM, human), ready for use	dark red	1 x 2.0 ml	
3. Positive control (IgM, human), ready for use	blue	1 x 2.0 ml	
4. Negative control (IgM, human), ready for use	green	1 x 2.0 ml	
5. Enzyme conjugate peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	
6. Sample buffer containing IgG/RF-Absorbent (Anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	
7. Wash buffer 10x concentrate	colourless	1 x 100 ml	
8. Chromogen/substrate solution TMB/H ₂ O ₂ , ready for use	colourless	1 x 12 ml	
9. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	
10. Test instruction	---	1 booklet	
11. Quality control certificate	---	1 protocol	
 Lot description			 Storage temperature
 In vitro diagnostic medical device			 Unopened usable until


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.



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.
- **Calibrator 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. The green coloured sample buffer contains IgG/RF absorbent. Serum or plasma samples diluted with this sample buffer are only to be used for the determination of IgM antibodies.
- **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.

Warning: The calibrator and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using. 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.

Introduction: Before the determination of specific antibodies of class IgM, antibodies of class IgG should be removed from the patient sample. This procedure must be carried out in order to prevent any rheumatoid factors of class IgM from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent specific IgG displacing IgM from the antigen, which would lead to false IgM-negative test results.

Functional principle: The sample buffer (green coloured!) contains an anti-human antibody preparation from goat. IgG from a serum sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

Separation properties:

- All IgG subclasses are bound and precipitated by the anti-human IgG antibodies.
- Human serum IgG in concentrations of up to 15 mg per ml are removed (average serum IgG concentration in adults: 12 mg per ml).
- Rheumatoid factors are also removed.
- The recovery rate of the IgM fraction is almost 100%.

Performance: The **patient samples** for analysis are diluted **1:101** with green coloured sample buffer. For example, add 10 µl sample to 1.0 ml sample buffer and mix well by vortexing. Sample pipettes are not suitable for mixing. Incubate the mixture for at least **10 minutes** at room temperature (+18°C to +25°C). Subsequently, it can be pipetted into the microplate wells according to the pipetting protocol.

Notes:

- Antibodies of the class IgG should not be analysed with this mixture.
- It is possible to check the efficacy of the IgG/RF absorbent for an individual patient sample by performing an IgG test in parallel to the IgM test using the mixture. If the IgG test is negative, the IgM result can be considered as reliable.
- The calibrator and controls are ready for use, do not dilute them.



Incubation

(Partly) manual test performance

<u>Sample incubation:</u> (1 st step)	Transfer 100 µl of the calibrator, 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).
<u>Washing:</u>	<p><u>Manual:</u> Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.</p> <p><u>Automatic:</u> Wash the reagent wells 3 times with 450 µl working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").</p> <p>Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual <u>and</u> 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.</p> <p><u>Note:</u> 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.</p> <p>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.</p>
<u>Conjugate incubation:</u> (2 nd step)	Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgM) into each of the microplate wells. Incubate for 30 minutes at room temperature (+18°C to +25°C).
<u>Washing:</u>	Empty the wells. Wash as described above.
<u>Substrate incubation:</u> (3 rd 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.
<u>Stopping:</u>	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.
<u>Measurement:</u>	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 enquiry.

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

The above pipetting protocol is an example of the **semiquantitative analysis** of antibodies in 24 patient samples (P 1 to P 24).

Calibrator (C), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.

The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises 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

The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (**cut-off**) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive, those below as negative.

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 calibrator. Use the following formula to calculate the ratio:

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

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	negative
Ratio ≥0.8 to <1.1:	borderline
Ratio ≥1.1:	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 IgG titer increases (exceeding factor 2) and/or seroconversion in a follow-up sample taken after at least 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-1, results are evaluated in the form of ratios which are a relative measurement of the antibody concentration in serum or plasma.

For every group of tests performed, the extinction values of the calibrator and ratios of 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 calibrator is 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 affinity purified glycoprotein C1 isolated from HSV-1. The purity of the preparation was verified by SDS polyacrylamid gelelectrophoresis. Glycoprotein C1 is a membrane protein which is only present in HSV-1 but not in HSV-2.

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 of the Anti-HSV-1 (gC1) ELISA (IgM) is ratio 0.04.

Cross reactivity: The quality of the antigen used ensures a high specificity of the ELISA. Sera from patients with other infections were investigated with the Anti-HSV-1 (gC1) ELISA (IgM).

Antibodies against	n	Anti-HSV-1 (gC1) ELISA (IgM) positive
Borrelia	10	0%
CMV	19	10.5%
EBV-CA	8	0%
Measles virus	13	0%
Mumps virus	11	0%
Rubella virus	10	0%
Toxoplasma gondii	14	0%
VZV	14	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 in 6 different test runs.

<i>Intra-assay variation, n = 20</i>		
Sample	Mean value (Ratio)	CV (%)
1	1.0	6.2
2	2.2	9.7
3	4.3	6.6

<i>Inter-assay variation, n = 4 x 6</i>		
Sample	Mean value (Ratio)	CV (%)
1	0.9	9.8
2	2.1	7.9
3	4.5	8.2

Sensitivity and specificity: 104 clinically precharacterised patient samples (INSTAND) were investigated with the EUROIMMUN Anti-HSV-1 (gC1) ELISA (IgM). The sensitivity amounted to 100% with a specificity of 100%. Borderline results were not included in the calculation.

n = 104		INSTAND		
		positive	borderline	negative
EUROIMMUN Anti-HSV-1 (gC1) ELISA (IgM)	positive	1	0	0
	borderline	0	0	2
	negative	0	0	101

The specificity of the Anti-HSV-1 (gC1) ELISA (IgM), was evaluated with 37 patient sera which were seropositive for rheumatoid factor. None of the in total 37 samples tested positive using the Anti-HSV-1 (gC1) ELISA (IgM).

Reference range: The levels of the anti-HSV-1 antibodies (IgM) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off ratio of 1.0, 0.8% of the blood donors were anti-HSV-1 positive (IgM).

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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