





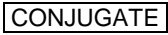









## Anti-HSV-1/2 Pool ELISA (IgM)

### Test instruction

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

**Principles of the test:** The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of the IgM class against HSV-1 and HSV-2 in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with a pool of HSV-1 and HSV-2 antigens. 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), which is capable of promoting a colour reaction.

#### Contents of the test kit:

Component	Colour	Format	Symbol
<b>1. Microplate wells</b> coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	
<b>2. Calibrator</b> (IgM, human), ready for use	dark red	1 x 2.0 ml	
<b>3. Positive control</b> (IgM, human), ready for use	blue	1 x 2.0 ml	
<b>4. Negative control</b> (IgM, human), ready for use	green	1 x 2.0 ml	
<b>5. Enzyme conjugate</b> peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	
<b>6. Sample buffer</b> containing IgG/RF-Absorbent (Anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	
<b>7. Wash buffer</b> 10x concentrate	colourless	1 x 100 ml	
<b>8. Chromogen/substrate solution</b> TMB/H <sub>2</sub> O <sub>2</sub> , ready for use	colourless	1 x 12 ml	
<b>9. Stop solution</b> 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	
<b>10. Test instruction</b>	---	1 booklet	
<b>11. Quality control certificate</b>	---	1 protocol	
<div style="display: flex; justify-content: space-between; align-items: center;"> <div>  Lot description   In vitro diagnostics         </div> <div style="text-align: center;">  </div> <div>  Storage temperature   Unopened usable until         </div> </div>			

**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 crystallization 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 deionized 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 controls and calibrators used have been tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid skin contact.

## Preparation and stability of the patient samples

**Sample material:** 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 a patient sample is tested for specific antibodies of the IgM class, antibodies of class IgG must be removed by ultracentrifugation, chromatography or immunoabsorption. This procedure must be carried out in order to prevent any rheumatoid factors 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 sample buffer. For example, add 10 µl serum to 1.0 ml sample buffer and mix well. Incubate the mixture for at least **10 minutes** at room temperature. Subsequently, it can be pipetted into the microplate wells according to the pipetting protocol.

**Notes:**

- Antibodies of the class IgG should not be analyzed 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 containing IgM antibodies are pre-diluted and ready for use, do not dilute them.



## Incubation

### (Partly) manual test performance

**Sample incubation:**  
(1<sup>st</sup> 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).

**Washing:** Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.  
Automatic: Wash reagent wells 3 times with 450 µl working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

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 reaction 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:**  
(2<sup>nd</sup> 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).

**Washing:** Empty the wells. Wash as described above.

**Substrate incubation:**  
(3<sup>rd</sup> 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 the reaction:** 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 of 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 the 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	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 minimizes 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

In cases of borderline test results, an additional patient sample should be taken 7 days later and re-tested in parallel with the first patient sample. The results of both samples allow proper evaluation of titer changes.

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For diagnosis, the clinical symptoms of the patient should always be taken into account along with the serological results.



## Test characteristics

**Calibration:** As no international reference serum exists for antibodies against HSV-1 and HSV-2, results are provided in the form of ratios which are a relative measure for the concentration of antibodies.

For every group of tests performed, the extinction values of the calibrator and the ratio 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 activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature during substrate incubation, 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 a pool of HSV-1 and HSV-2 antigens. The antigen source is provided by inactivated cell lysates of cells infected either with the "Mac Intyre" strain (ATCC VR-539) of Herpes-simplex-1 virus or with the "G" strain (ATCC VR-734) of Herpes-simplex-2 virus.

**Detection limit:** The lower detection limit is defined as a mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The detection limit of the Anti-HSV-1/2 Pool ELISA (IgM) is ratio 0.05.

**Cross reactivity:** The quality of the antigen used ensures high specificity of the ELISA. However, cross reactions with other herpes viruses cannot be excluded. Samples from patients with acute infections caused by different agents were investigated using the Anti-HSV-1/2 Pool ELISA (IgM).

Antibodies against	n	Anti-HSV-1/2 Pool ELISA (IgM)
<b>Borrelia</b>	10	0%
<b>CMV</b>	10	0%
<b>EBV-CA</b>	10	0%
<b>Measles virus</b>	8	12.5%
<b>Mumps virus</b>	10	30.0%
<b>Parvovirus B19</b>	10	10.0%
<b>Rubella virus</b>	10	10.0%
<b>TBE virus</b>	10	0%
<b>Toxoplasma gondii</b>	10	10.0%
<b>VZV</b>	5	20.0%

**Interference:** Haemolytic, lipaemic and icteric samples showed no influence at the result up to a concentration of 10 mg/ml for hemoglobin, 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 using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed on 6 different days.

<i>Intra-assay variation, n = 20</i>		
Serum	Mean value (Ratio)	CV (%)
1	0.9	10.4
2	1.0	6.1
3	1.8	9.7

<i>Inter-assay variation, n = 4 x 6</i>		
Serum	Mean value (Ratio)	CV (%)
1	1.0	9.1
2	1.2	8.2
3	1.9	9.1



**Specificity and sensitivity:** Samples from 284 patients (origin: USA and Canada) were investigated using the EUROIMMUN Anti-HSV-1/2 Pool ELISA and another commercially available ELISA. The EUROIMMUN ELISA showed a specificity of 93.1% and a sensitivity of 95.7% with reference to the comparison ELISA.

n = 284		Comparison ELISA		
		positive	borderline	negative
<b>EUROIMMUN Anti-HSV-1/2 Pool ELISA (IgM)</b>	positive	67	12	10
	borderline	4	20	20
	negative	3	13	135

Furthermore 55 clinically characterized patient samples (Interlaboratory test samples from INSTAND, Germany) were examined with the EUROIMMUN Anti-HSV-1/2 Pool ELISA (IgM). The EUROIMMUN ELISA showed a specificity and a sensitivity of 100% each.

n = 55		INSTAND		
		positive	borderline	negative
<b>EUROIMMUN Anti-HSV-1/2 Pool ELISA (IgM)</b>	positive	1	0	0
	borderline	0	0	0
	negative	0	0	54

Borderline samples were not included in the calculation of specificity and sensitivity.

**Reference range:** The levels of anti-HSV-1/2 antibodies (IgM) were analyzed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off ratio of 1.0, all of the blood donors were anti-HSV-1/2 negative (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 herpes virus 1, HHV-1) and/or herpes simplex virus 2 (HSV-2, human 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 [1, 2, 3].

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 [1, 3, 4].

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 [5, 6, 7, 8]. 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 per second [9].

In primary HSV-2 infections, cephalalgia and neck stiffness are frequent symptoms, whereas meningitis is rarely observed [1]. 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 [1, 3, 4, 7, 10]. For this reason caesarean section is indicated in acute infections, regardless of primary infection or relapse [11]. 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 [12].

The seroprevalence increases up to the age of 50 [2, 13]. Provocable relapses are typical [12]. 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%-90% [2]. Antibodies against HSV-2 can be found in only 7%-20% of general population and in more than 20% of promiscuous adults [2, 7, 8]. Mixed infection with both types is revealed in about 11% [14]. Parallel infections of HIV-1 and HSV-2 have been observed in 95% of herpes simplex patients in Africa [5, 6, 7]. HSV infections are the most frequent co-infections found in HIV-infected persons [16]. HSV-2 micro and macro ulcerations in the genital mucosa can promote further infection [5, 6, 7, 8, 16].

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 [10, 11, 17]. 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 [1, 14]. PCR is the method of choice for early detection of HSV infections during pregnancy, particularly to determine the necessity of caesarean section [11, 18]. For timely diagnosis of HSV encephalitis the determination of HSV DNA in CSF using PCR is indispensable [18]. 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 [3, 20]. 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 [21, 22, 23].

Type-specific serological diagnosis of herpes simplex virus infections requires test systems based on type-specific antigens, namely glycoprotein C-1 (gC-1) or glycoprotein G-1 (gG-1) of HSV-1 and glycoprotein G-2 (gG-2) of HSV-2 [12, 24, 25, 26, 27]. 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 [12, 13, 24, 25, 28, 29, 30, 31]. 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 [12, 27].





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