Herpes simplex virus 1/2 IgM ELISA

Enzyme immunoassay for the qualitative determination of IgM class antibodies against Herpes simplex virus 1/2 in human serum or plasma (citrate, heparin).

REF RE57951

Σ 96

2-8°C

EU: IVD CE
1. INTRODUCTION

Herpes simplex is an enveloped DNA virus (150-200 nm in diameter) belonging to the alpha-herpesviridae. Based on antigenic, biochemical and biologically differences it can be divided into two serotypes, HSV-1 and HSV-2. Man is the only known natural host and source of the virus. HSV Type 1 typically causes oral herpes, while HSV-type 2 typically affects the genital area. Most of the time, HSV-1 and HSV-2 are inactive, or "silent", and cause no symptoms, but some infected people have "outbreaks" of blisters and ulcers. Once infected with HSV, people remain infected for life. Herpes simplex viruses are amongst the most common infectious agents of man, and either HSV type appears to be capable of infecting similar body sites. A high percentage of the adult population is seropositive (approx. 90% HSV-1, in dependence on the socioeconomic status 10-30% HSV-2). Primary HSV-1 infection usually occurs in early childhood (6 to 18 months of age). HSV-2 usually produces mild symptoms, and most people have no recognized symptoms. Persons at risk are children with inherited T-cell deficiencies and patients who are immunosuppressed because of infection (e.g. HIV), transplantation, or cancer therapy.

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
<th>Symptoms (e.g.)</th>
<th>Transmission route</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
<td>Oral herpes (herpes labialis)</td>
<td>Fever, sore throat, lymphadenopathy, multiple vesicles in the oral/genital mucous membranes, gingivostomatitis</td>
<td>Transmission by droplet infection; Sexually transmitted disease (STD); Perinatal; Congenital</td>
</tr>
<tr>
<td>HSV-2</td>
<td>Genital herpes (herpes genitalis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neonatal herpes (Herpes neonatorum)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The presence of pathogen or infection may be identified by
- Microscopy
- PCR
- Serology: e.g. by ELISA

2. INTENDED USE

The Herpes simplex virus 1/2 IgM ELISA is intended for the qualitative determination of IgM class antibodies against Herpes simplex virus 1+2 (HSV) in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbert Assay) technique. Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.
4. MATERIALS

4.1. Reagents supplied

- **MTP** Herpes Simplex Virus 1+2 (HSV) Coated Microplate (IgM): 12 break-apart 8-well snap-off strips coated with Herpes Simplex Virus 1+2 (HSV) antigens; in resealable aluminium foil.

- **SAMPLEDIL** IgM Sample Diluent: 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap.

- **STOP** Stop Solution: 1 bottle containing 15 mL sulphuric acid, 0.2 mol/l; ready to use; red cap.

- **WASHBUF CONC** Washing Buffer (20x conc.): 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.

- **ENZCONJ** Herpes Simplex Virus 1+2 (HSV) anti-IgM Conjugate: 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap.

- **TMB SUBS** TMB Substrate Solution: 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap; < 5 % NMP.

- **CONTROL +** Herpes Simplex Virus 1+2 (HSV) IgM Positive Control: 1 vial containing 2 mL control (human serum or plasma); coloured yellow; ready to use; red cap.

- **CONTROL CO** Herpes Simplex Virus 1+2 (HSV) IgM Cut-off Control: 1 vial containing 3 mL control (human serum or plasma); coloured yellow; ready to use; green cap.

- **CONTROL -** Herpes Simplex Virus 1+2 (HSV) IgM Negative Control: 1 vial containing 2 mL control (human serum or plasma); coloured yellow; ready to use; blue cap.

For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2 - 8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2 - 8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20 - 25 °C) and mix them before starting the test run!

6.1. Coated Microplate

The break-apart snap-off strips are coated with Herpes Simplex Virus 1+2 (HSV) antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2 - 8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20 - 25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.
6.3. TMB Substrate Solution
The reagent is ready to use and has to be stored at 2 - 8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION
Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2 - 8 °C; otherwise they should be aliquoted and stored deep-frozen (-70 to -20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

7.1. Sample Dilution
Before assaying, all samples should be diluted 1+100 with IgM Sample Diluent. Dispense 10 µL sample and 1 mL IgM Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

8.1. Test Preparation
Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout. Select the required number of microtiter strips or wells and insert them into the holder. Perform all assay steps in the order given and without any delays. A clean, disposable tip should be used for dispensing each standard/control and sample. Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20 - 25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20 - 25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.2. Measurement
Adjust the ELISA microwell plate reader to zero using the Substrate Blank.
If - due to technical reasons - the ELISA microwell plate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!
Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard/control and sample in the-plate layout.
Bichromatic measurement using a reference wavelength of 620 nm is recommended. Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria
In order for an assay to be considered valid, the following criteria must be met:

- **Substrate Blank**: Absorbance value < 0.100
- **Negative Control**: Absorbance value < 0.200 and < Cut-off
- **Cut-off Control**: Absorbance value 0.150 – 1.300
- **Positive Control**: Absorbance value > Cut-off

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results
The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43

Cut-off = 0.43

9.2.1. Results in Units [U]
Sample (mean) absorbance value x 10 = [Units = U] / Cut-off

Example: $1.591 \times 10 = 37 \text{ U (Units)}$ / 0.43

9.3. Interpretation of Results

<table>
<thead>
<tr>
<th>Cut-off</th>
<th>10 U</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>&gt; 11 U</td>
<td>Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).</td>
</tr>
<tr>
<td>Equivocal</td>
<td>9 – 11 U</td>
<td>Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative.</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt; 9 U</td>
<td>The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.</td>
</tr>
</tbody>
</table>

Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.

In immunocompromised patients and newborns serological data only have restricted value.
9.3.1. Antibody Isotypes and State of Infection

<table>
<thead>
<tr>
<th>Serology</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM</td>
</tr>
<tr>
<td>IgG</td>
<td>Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection</td>
</tr>
</tbody>
</table>

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications. For further information about the specific performance characteristics please contact IBL International GmbH.

10.1. Precision

<table>
<thead>
<tr>
<th>Intraassay</th>
<th>n</th>
<th>Mean (E)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>24</td>
<td>0.588</td>
<td>9.47</td>
</tr>
<tr>
<td>#2</td>
<td>24</td>
<td>1.105</td>
<td>5.12</td>
</tr>
<tr>
<td>#3</td>
<td>24</td>
<td>1.630</td>
<td>1.97</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interassay</th>
<th>n</th>
<th>Mean (U)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>12</td>
<td>22.02</td>
<td>2.18</td>
</tr>
<tr>
<td>#2</td>
<td>12</td>
<td>34.21</td>
<td>6.93</td>
</tr>
<tr>
<td>#3</td>
<td>12</td>
<td>1.70</td>
<td>12.25</td>
</tr>
</tbody>
</table>

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 98.49% (95% confidence interval: 95.66% - 99.69%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100.0% (95% confidence interval: 39.76% - 100.0%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal significant evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.
12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

12.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: RE57951 Herpes simplex virus 1/2 IgM ELISA


**ABBREVIATIONS**

| NMP | N-Methyl-2-pyrrolidone |
SUMMARY OF TEST PROCEDURE

SCHEME OF THE ASSAY
Herpes simplex virus 1/2 IgM ELISA

**Test Preparation**

<table>
<thead>
<tr>
<th></th>
<th>Substrate Blank (A1)</th>
<th>Negative Control</th>
<th>Cut-off Control</th>
<th>Positive Control</th>
<th>Sample (diluted 1+100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>-</td>
<td>100 µL</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cut-off Control</td>
<td>-</td>
<td>-</td>
<td>100 µL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 µL</td>
<td>-</td>
</tr>
<tr>
<td>Sample (diluted 1+100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

Cover wells with foil supplied in the kit

**Incubate for 1 h at 37 °C**
Wash each well three times with 300 µL of Washing Buffer

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>-</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
</table>

**Incubate for 30 min at room temperature (20 - 25 °C)**
Do not expose to direct sunlight
Wash each well three times with 300 µL of Washing Buffer

<table>
<thead>
<tr>
<th>TMB Substrate solution</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
</table>

**Incubate for exactly 15 min at room temperature (20 - 25 °C) in the dark**

<table>
<thead>
<tr>
<th>Stop Solution</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
</thead>
</table>

Photometric measurement at 450 nm
(reference wavelength: 620 nm)
<table>
<thead>
<tr>
<th>REF</th>
<th>Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote n.: / Αριθμός-Παραγωγή:</td>
</tr>
<tr>
<td></td>
<td>Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:</td>
</tr>
<tr>
<td>CONC</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα</td>
</tr>
<tr>
<td>LYO</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Λυοφιλιασμένο</td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamiento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Επιμελούση.</td>
</tr>
<tr>
<td></td>
<td>Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.</td>
</tr>
<tr>
<td></td>
<td>Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l’abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Να φυλάσσεστε μακριά από θερμοκρασία και άμεση επαφή με το φως του ηλίου.</td>
</tr>
<tr>
<td></td>
<td>Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:</td>
</tr>
<tr>
<td></td>
<td>Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:</td>
</tr>
</tbody>
</table>

**COMPLAINTS:** Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

**WARRANTY:** The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

**LIMITATION OF LIABILITY:** IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER’S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.