HSV IgM ELISA Kit

Prod. No.: DEIA547
Pkg. Size: 96T

INTENDED USE

ELISA test for detection of IgM antibodies to Herpes simplex virus in human serum or plasma.

GENERAL DESCRIPTION

Herpes Simplex virus (HSV) belongs to the Herpes virus group which also includes Cytomegalovirus (CMV), Varicella Zoster Virus (VZV) and Epstein-Barr Virus (EBV). A common characteristic of these viruses is that they can cause latent infections. HSV is among the most common infectious agents of man. The clinical manifestations vary, but may be more severe in certain types of hosts, for instance the newborn or immunocompromised patient and with involvement of certain sites such as the central nervous system. HSV can cause genital, neurological, oral and respiratory infections and is the causative agent of labial herpes, herpetic keratitis and herpes of the skin. Serological diagnosis of HSV infections can be performed by testing for a significant rise in HSV-specific IgG antibodies in paired sera and for HSV-specific IgM antibodies. In general, it is advised to test a combination of IgG and IgM. Specific IgM antibodies are indicative of primary HSV infection and appear usually within the first week after onset of illness. An IgM antibody response also often follows severe secondary herpetic infections, such as meningoccephalitis or eczema herpeticus, but in connection with the appearance of ordinary recurrent orofacial or genital lesions specific IgM antibodies are usually not detectable. Crossreactivity between HSV and VZV may exist and an interpretation of HSV serological results should be related with clinical data. Specific IgG antibody response can be detected by the use of serum pairs in primary or recurrent HSV infections. This assay makes use of whole virus based HSV type 1 antigen. Crossreactivity with HSV-2 antigen may occur. Serological response to HSV may consist of reactivity to HSV common antigens and to type specific antigens. Whole virus based assays can not give reliable data for identifying a type 1 or type 2 infection. Therefore type specific interpretation of results must be done with great care.

PRINCIPLE OF THE TEST

CD HSV IgM is an IgM-capture immunoenzymatic assay that is performed by incubating diluted test specimens in microplate wells coated with rabbit antibodies specific for the μ-chain of human IgM. All the IgM class antibodies present in the sample will bind to the solid-phase antibody. Subsequently, the wells are washed to remove residual test specimen, and HSV-1 antigen conjugated with peroxidase (HRPO) is added. HSV-1 antigen is prepared by extraction and purification of Herpes simplex virus propagated in HEP-2 cells. To minimize unspecific reactivity, unlabelled control antigen consisting of uninfected cellular components is added to the conjugate. The HSV-HRPO conjugate will bind to the captured HSV-specific IgM. After another washing to eliminate unbound material a solution of enzyme substrate and chromogen is added. This solution will develop a blue colour if the sample contains anti-HSV IgM. The blue colour changes to yellow after blocking the reaction with sulphuric acid. The intensity of the colour is proportional to the amount of anti-HSV IgM in the test specimens.

REAGENTS PROVIDED

1. MICROPLATE: 12 x 8 well strips coated with rabbit anti-human IgM. Individually separable wells.
2. CONCENTRATE CONJUGATE: 1 x 250 μl of HSV-1 antigen conjugated with peroxidase in phosphate buffer containing bovine serum albumin and preservatives.
3. CONTROL ANTIGEN: 1 x 250 μl of sonicated HEP-2 cells in phosphate buffer containing bovine serum albumin and preservatives.
4. CONJUGATE DILUENT: 1 x 20 ml of phoshate buffer containing proteins, blue dye and preservatives. Ready to use.
5. SAMPLE DILUENT: 1 x 120 ml of phosphate buffer containing stabilisers protein, blue dye, and preservatives. Ready to use.
6. WASHING SOLUTION: 1 x 100 ml of concentrate phosphate buffer (10x) containing Tween 20 and preservatives. To be diluted 1/10 in distilled or deionised water before use.
7. SUBSTRATE BUFFER: 1 x 14 ml of citrate-acetate buffer containing hydrogen peroxide. Ready to use.
8. CHROMOGEN: 1 x 1.5 ml of 3,3',5,5’-Tetramethylbenzidine (TMB) dissolved in dimethylsulphoxide (DMSO).
9. HIGH POSITIVE CONTROL: 1 x 1.0 ml of diluted human serum containing IgM antibodies to HSV. Contains bovine serum albumin, preservatives and red dye. Ready to use.
10. LOW POSITIVE CONTROL: 1 x 1.8 ml of diluted human serum containing a low level of IgM antibodies to HSV. Contains bovine serum albumin, preservatives and green dye. Ready to use.
11. NEGATIVE CONTROL: 1 x 1.0 ml of diluted human serum negative for anti-HSV IgM. Contains bovine serum albumin, preservatives and yellow dye. Ready to use.
12. STOPPING SOLUTION: To cover the microplate during incubations.
13. ADHESIVE SEALS: For storage of unused strips.
14. RESEALABLE BAG: For storage of unused strips.

STORAGE AND STABILITY

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The components will remain stable through the expiration date shown on the label if stored between 2-8°C. The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells. Once opened the bag, unused strips are stable for 3 months if stored at 2-8°C tightly sealed in the plastic bag provided, with the silicagel included. Once diluted, the washing solution is stable for one week if stored at room temperature or one month at 2-8°C. Once diluted for using, the conjugate should be used immediately. Store the chromogen in the dark. Once prepared for using, the substrate-TMB is stable for 8 hours at room temperature (20-25°C).

SPECIMEN COLLECTION AND HANDLING

Use fresh serum or plasma (EDTA). Other anticoagulants should be evaluated before use. Samples can be stored at 2-8°C for 3 days. For longer periods, samples should be frozen (-20°C). Avoid repeated freezing and thawing. Samples showing visible particulate matter should be clarified by centrifugation. Serum or plasma samples should not be heat inactivated, since that may cause incorrect results.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionised water.
2. Multichannel pipettes and micropipettes (10 µl to 1000 µl) and disposable tips.
3. Incubator at 37°C ± 1°C.
4. Tubemicrotubes for dilutions.
5. Timer.
6. Microplate reader with a 450 nm filter. Reference filter of 620 or 630 nm is advisable.
7. Manual or automated wash system.

ASSAY PROCEDURE

1. Reagent And Sample Preparation
   1) Allow all the reagents to reach room temperature (20-25°C) before running the assay.
   2) Gently mix all liquid reagents before use.
   3) Dilute the concentrate washing solution 1/10 with distilled or deionised water. For one plate, mix 50 ml of the concentrate solution with 450 ml of water. If less than a whole plate is used, prepare the proportional volume of solution.
   4) Prepare 1/101 dilution of test samples by adding, for example, 10 µl of sample to 1 ml of sample diluent. Mix well. DO NOT DILUTE CONTROLS; THEY ARE READY TO USE.
2. Assay Steps
   1) Use only the number of strips required for the test. Reserve 9 wells for blank and controls. Use two wells for the negative and high positive controls and four wells for the low positive control. Pipette 100 µl of each control and of each diluted sample to the corresponding wells. Leave a well empty for the substrate blank.
   2) Cover the microplate with an adhesive seal, or place in a 100% moist atmosphere, and incubate for 1 hour at 37°C.

3) During the last 15 minutes of this incubation prepare the working conjugate as follows: for each 8 wells strip pipette 1 ml of conjugate diluent, 10 µl of conjugate and 10 µl of control antigen. Mix well.
4) Remove and discard the adhesive seal. Aspirate the contents of the wells and fill them completely (approximately 350 µl) with the diluted washing solution. Repeat the process of aspiration and washing 4 more times. Ensure that each column of wells soak for at least 15 seconds before the next aspiration cycle.
5) Transfer 100 µl of the conjugate diluted as described in step 3, to each well except the one for the substrate blank. Avoid bubbles upon addition.
6) Cover the microplate with an adhesive seal, or place in a 100% moist atmosphere, and incubate for 1 hour at 37°C.
7) Prepare the substrate-chromogen solution following the table 1. The final solution should be colourless; discard if it becomes blue.

Table 1:

<table>
<thead>
<tr>
<th>Strips required</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate buffer ml</td>
<td>0.9</td>
<td>1.8</td>
<td>3.6</td>
<td>5.4</td>
<td>7.2</td>
<td>9.0</td>
<td>10.8</td>
</tr>
<tr>
<td>Chromogen (TMB) µl</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
<td>1.0</td>
<td>1.2</td>
</tr>
</tbody>
</table>

8) Remove and discard the adhesive seal. Aspirate and wash the wells as in step 4.
9) Add 100 µl of substrate-TMB solution to each well, including the blank.
10) Incubate for 30 minutes at room temperature (20-25°C).
11) Stop the reaction by adding 100 µl of stopping solution in the same sequence and time intervals as for the substrate-TMB.
12) Blank the reader at 450 nm with the blank well and read the absorbance of each well, within 30 minutes. It is recommended to read in bichromatic mode using a 620 - 630 nm reference filter.

RESULTS

1. Calculate the mean absorbance value of the negative and positive controls. The cut-off value is: Cut-off = LPCX
2. Divide the sample absorbance by the cut-off value.
   Positive: ratio absorbance/cut-off ≥ 1.1
   Negative: ratio absorbance/cut-off < 0.9
   Equivocal: ratio absorbance/cut-off ≥ 0.9 < 1.1

INTERPRETATION OF RESULTS

A positive reaction should be interpreted as presence of HSV IgM antibodies and infection by Herpes Simplex virus. Following a primary infection, IgM class antibody appears usually within the first week and generally lasts for no more than 4 months in the normal individual.

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In secondary infections as reactivation of latent virus or reinfection, some patients develop IgM while others do not. For diagnosis purpose, the detection of IgM antibodies to HSV should be associated with the study of specific IgG seroconversion by testing paired samples taken at 2-3 weeks interval and the detection of virus DNA in white blood cells or in total blood by amplification techniques.

**ASSAY CHARACTERISTICS**

1. Evaluations
The performance of CD HSV IgM has been evaluated at an independent Dutch Public Health Laboratory.
   a. The diagnostic specificity was assessed by testing 34 samples selected from a population consisting of 34 negative for HSV IgM. Out of 34 samples tested, the kit proved to be negative in 33 cases, giving a diagnostic specificity of 97% (33/34).
   b. The diagnostic sensitivity was assessed by testing 30 samples obtained from patients with a HSV infection in progress. 24 of these samples resulted positive; therefore the diagnostic sensitivity is 80% (24/30).

2. Precision
Different samples containing different levels of the parameter determined were assayed to assess repeatability and reproducibility of the test (intra-assay and inter-assay variability). Computed results on these samples give coefficients of variation values lower than 10%.

3. Interferences
Controlled studies of potentially interfering substances or conditions showed that the assay performance was not significantly affected by either anticoagulants (EDTA), slight hemolysis or freezing.

**QUALITY CONTROL**

Results of an assay are valid if the following criteria are accomplished:
1. Substrate blank: absorbance value must be less than or equal to 0.100.
2. Low positive control: each of the individual absorbance values must not differ more than 25% of the mean of the four values. The mean absorbance must be between 0.200 and 0.600 after subtracting the blank.
3. Ratio negative control/low positive control less than 0.7.
4. Ratio high positive control/low positive control greater than 2.0.

**REFERENCES**