

HSV1,2 IgM ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the Detection of
Herpes simplex virus 1, 2 IgM Antibody
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-HSV1, 2 IgM-96

PISHTAZ TEB DIAGNOSTICS

Introduction

Herpesviridae family includes large number of DNA viruses which known as one of the most common cause of human infection worldwide. *Herpes simplex virus* (HSV) infection caused by two virus types known as HSV1 and HSV2. HSV-1 mostly causes non genital lesions but HSV-2 commonly infects genital organs. Infection caused by Herpes simplex virus type I known as nongenital warts (cold sore) occurs mostly in young children (less than 5 years old in 90% of cases). It affects lips, gingiva, mouth, occasionally cornea and sometimes genital area with very small painful vesicles. In the case of eye infection, painful red eye, feeling of presence of foreign materials in eyes, photosensitivity and uncontrolled tears may be manifested. Direct person to person as well as contact with infected human secretions like saliva, tears, urine and feces are major routes of HSV transmission. Unhealed vesicles are also contagious and contact with their secretion may transmit the virus. HSV2 mostly cause genital lesions in adults and accompanied with sexual activity. HSV infections signs and symptoms appears 4-7 days after contact with infected individual. In most of cases the disease is asymptomatic particularly in pregnant and healthy individuals. Immunocompromised patients or patients under immunosuppressive therapy are affected more frequently. HSV cause very small vesicles in genital area which became extremely painful after rupture. In some affected females, vaginal secretion may appear. HSV neural involvement may provoke fever, headache, vomiting and neck stiffness 3-12 days after genital lesions. In addition to sexual contact, skin contact with respiratory secretion of infected individual can also transmit the infection. Consequently, certain practitioners like dentists, medical staffs and laboratory personnel are at high risk of acquiring infection. Congenital transmission during child birth is also an important route of HSV infection transmission.

IgM antibodies against HSV1, 2 antigens raise in early infection and then declined and replaced by IgA and IgG antibodies. Detection of specific IgM antibody against HSV antigens is used for the detection of acute infection.

Test Principle

The test principle is based on indirect ELISA technique in which diluted patient serum samples are allowed to react with coated HSV1,2 antigens. After incubation, the HRP -conjugated anti-human IgM is added into the wells. If anti-HSV1,2 IgM was present in samples, they will react with the HRP-conjugated anti-human IgM. After incubation and appropriate washing, a solution of chromogen substrate is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of HSV1, 2 IgM is directly proportional to the color intensity of the test sample.

Materials Provided With Kit

1. Antigen coated plate (1 plate, 96 wells): Microtiter wells coated with HSV1,2 antigens.
2. Sample diluent: 1 vial contains 50 ml of diluent to dilute samples. The solution contains buffer, protein and stabilizer.
3. Assay buffer: 1 vial contains 7.5 ml of anti human IgG to block rheumatoid factor and high IgG concentration in serum.
4. Conjugate solution: 1 vial, 12 ml volume. The solution contains HRP conjugated anti-human IgM, buffer and preservative. Ready to use.
5. Positive control serum: 1 vial contains 1.5 ml of buffer solution, protein as stabilizer, 0.05% Kathon CG as preservative and inactive human serum contains anti-HSV 1,2 IgM antibody.
6. Negative control serum: 1 vial contain 2 ml of phosphate buffer, 0.05% Kathon CG as preservative and human serum negative for anti-HSV 1,2 IgM antibody.



7. Wash solution (20X): 1 vial, 50 ml, Phosphate Buffered Saline (PBS) containing 0.05 % Tween 20 as detergent and Kathon CG as preservative.
8. Chromogen-substrate: 1 vial, 12 ml, contains tetramethyl benzidine (TMB) and hydrogen peroxide, ready to use.
9. Stop solution: 1 vial, 12 ml, 1 molar hydrochloric acid solution.
10. Cardboard sealer.

Materials/Equipment required but not provided with Test Kit

1. ELISA reader with 450 and 630 nm (reference) filters.
2. Precision pipettes
3. Distilled water
4. Incubator or water bath (37°C)
5. Absorbent paper

General Information

1. Do not mix kit reagents from different lot numbers. All kit components must be used only in original kit.
2. This kit is just for the detection of Anti-HSV 1,2 IgM in human serum and plasma.
3. All reagents obtained from human sources are negative for HIV Ag, HIV and HCV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Opened microplates are stable for 4 months.
4. Reagents stability period are marked on their labes.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate the specimen (maximum 2 days at 2-8°C) and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C. Avoid freeze-thaw of specimen during storage. Samples suspected to microbial contamination should not be used.

Reagents & Specimens Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: Warm the vial at 37°C to dissolve possible crystals which formed due to concentration of solution. dilute concentrated wash solution 1:20 with distilled water before use. This solution will be stable for 1 week at 2-8°C.
3. Dilute samples 1:51 with sample diluent (dilute 10 µl of sample with 500 µl of sample diluent).
4. In a serperate tube, to 75 µl of each diluted sera add 75 µl of assay buffer, mixed well and incubate for 10 minutes at room temperature.

Note: Control sera are ready to use and do not need any dilution.

Assay Procedure

1. Use required number of wells and keep the remaining with desiccants in tightly closed sealed bag. Test steps should be done sequentially.

1. Add 100 µl of each control as well as diluted test sera into appropriate wells. Consider two consecutive wells in first strip as **Blank**, **Positive** respectively, next two wells for duplicate **Negative control serum**. Rest of wells are used for diluted samples. Test steps should be done sequentially.
2. Seal the plate with cardboard sealer tightly and leave wells for 30 minutes at 37°C room temperature.
3. Remove the wells content by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each with 300 µl of Working wash solution).
4. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
5. Add 100 µl of ready to use conjugate solution into all wells except blank.
6. Seal the plate with cardboard sealer tightly. Mix gently and incubate wells for 30 minutes at 37°C.
7. Repeat steps of 3 and 4.
8. Spike 100 µl of chromogen/substrate solution to each well.
9. Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
10. Add 100 µl of stop solution to the wells to stop reaction.
11. Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available). Reference filter is highly recommended.
2. The OD value for the **Negative control serum** is lower than 0.15. Higher values indicate an inappropriate washing procedure. In such a case, check the efficiency of the washing device.
3. The OD value of **Positive control serum** is higher than the 0.6. Lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

Result Calculation

1. Measure absorbance of controls and samples at 450 nm (Use 630 nm filter as reference filter if it's available).
2. Reduce blank OD from tests and controls OD.
To estimate Cut off value, Mean OD of Cut off control sera should be calculated:

$$\text{Cut-off value} = \text{Mean OD of negative control serum} + 0.15$$

To distinguish between positive and negative results the cut off index should be determined:

$$\text{Cut-off index} = \text{OD of sample} / \text{Cut-off value}$$

Based on above formula, Results lower than 0.9 are considered as negative and those greater than 1.1 considered as positive results. Those results between 0.9-1.1 are considered as suspected results and should be re-evaluated with fresh samples after a while.

Validity of the Assay

The assay is to be considered valid if:

1. The OD (450 nm) value of the **Blank** should be lower than 0.1. Higher values indicate chromogen/substrate contamination.

Results Evaluation

1. Negative results indicate absence of anti-HSV 1,2- IgM antibody.
2. Positive results should be rechecked and those which become negative should be reported as negative. Faulty



washing and sampling errors may leads to first positive results.

Performance Characteristics

Sensitivity

To evaluate test sensitivity, 60 positive samples confirmed by chemiluminescence and ELISA were used which among them 59 sample showed positive results. The PT-HSV1,2-IgM-ELISA showed 98% sensitivity which is comparable to other diagnostic kits or methods.

Specificity

To evaluate test specificity, 500 negative sera are tested simultaneously with PT-HSV1,2-IgM-ELISA and chemiluminescence method. Results displayed all tests were negative. According to test results the PT-HSV1,2-IgM-ELISA showed 100% specificity.

Test Precision

To verify test repeatability intra- and inter assay tests were performed on positive, weakly positive and negative sera. Results are shown below:

Intra-assay test results

| | Number of Repeats | OD Mean | SD | CV% |
|-------------------------|-------------------|---------|-------|-------------|
| Positive Sample | 20 | 1.0 | 0.13 | 13.0 |
| Negative Samples | 20 | 0.05 | 0.003 | 6.0 |
| Weak Positive | 20 | 0.23 | 0.013 | 5.6 |

Inter-assay test results

| | Number of Repeats | OD Mean | SD | CV% |
|-------------------------|-------------------|---------|-------|------|
| Positive Sample | 10 | 1.1 | 0.14 | 12.7 |
| Negative Samples | 10 | 0.05 | 0.004 | 8.0 |
| Weak Positive | 10 | 0.24 | 0.02 | 8.3 |

All test were run in duplica

References

1-Mahy B.W.J and Meulen V.T. (2005). Topley and Wilson's Microbiology and Microbial Infections: Virology. Volume 2. Tenth edition. London. Hodder Arnold.




2-Lennette E.H. and Smith T.F. (1999). Laboratory diagnosis of viral infections. Third edition. New York. Marcel Dekker.

3-Connie R.M. and Manuselis G. (2000). Text book of diagnostic microbiology. Second edition. Philadelphia. W.B. Saunders.

4-Major M.E., Rehermann B. and Feinstone S.M. (2001). Fields Virology. Fourth edition. Philadelphia. Lippincott Williams and Wilkins.

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| Schematic HSV1,2-IgM ELISA Test Procedure |
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Step 1




| Reagent | Control Serum | Blank | Sample |
|-------------------------|---|---|---|
| |  |  |  |
| Control Sera | 100 µl | None | None |
| Prepared Diluted Sample | None | None | 100 µl |

Cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at 37°C.


Step 2

Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.

Step 3




| | | | |
|---------------------------|---|---|---|
| Enzyme Conjugate Solution | 100 µl  | None  | 100 µl  |
|---------------------------|---|---|---|

Cover the microplate wells with cardboard sealer and incubate for 30 minutes at 37°C.


Step 4




Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.


Step 5

| | | | |
|------------------------------|---|---|---|
| Chromogen Substrate Solution | 100 µl  | 100 µl  | 100 µl  |
|------------------------------|---|---|---|

Incubate wells for 15 minutes at room temperature and dark.


Step 6

| | | | |
|---------------|---|---|---|
| Stop Solution | 100 µl  | 100 µl  | 100 µl  |
|---------------|---|---|---|

Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).