

Human Herpes Simplex Virus TypeI (HSV1) IgM ELISA Kit

Catalog No: E-HD-E101

96T

Version Number:	V1.1
Replace version:	V1.1
Revision Date:	2026.01.21

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This ELISA kit uses Capture-ELISA as the method to detect the HSV-I-IgM in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with Mouse-anti-human IgM (μ chain). Samples are added to the ELISA Microtiter plate wells and the IgM antibody in which will be captured. Free components are washed away (including the specific IgG antibody). The HRP conjugate (HRP conjugated HSV-I-antigen) is added to each well and incubate. The “HRP conjugated HSV-I-antigen” will react with the “anti- μ chain-HSV-I- IgM antibody” compound to form “anti- μ chain- HSV-I-IgM antibody -HRP conjugated HSV-I-antigen” compound. The TMB substrate is added after washing to initiate the color developing reaction. The presence of HSV-I-IgM antibody can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

Kit components

Item	Specifications
ELISA Microtiter plate	96T
Positive Control	1 mL
Negative Control	1 mL
Sample Diluent	12 mL
HRP Conjugate	12 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Plate Sealer	1 piece
Sealed Bag	1 piece
Manual	1 copy

Experimental instrument

Micro-plate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)

High-precision transferpettor, EP tubes and disposable pipette tips

37°C Incubator or water bath

Deionized or distilled water

Absorbent paper

Loading slot for Wash Buffer

Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
3. The Stop Solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. FOR RESEARCH USE ONLY. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The tested sample should be kept fresh.
7. The results shall depend on the readings of the Micro-plate Reader.
8. **Each reagent is optimized for use in the E-HD-E101. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E101 with different lot numbers.**
9. Do not use components from different batches of kit.

Storage and expiry date

Store unopened at 2 to 8°C. Do not freeze.

Please store the opened kit at 2-8°C, protect from light and moisture. The shelf life of the opened kit is up to 1 months.

Expiry date: expiration date is on the packing box.

Sample preparation

1. Use the conventional method to collect the serum sample. Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. The suspended fibrous protein may cause a false positive if not fully precipitated. Adjust the samples to room temperature (30 minutes) before use. Frozen sample should be adjusted to room temperature and mixed fully.
2. Anticoagulants (heparin, EDTA, sodium citrate) has no interference to the test results. In general, endogenous interferent (such as blood lipids, cholerythrin, hemoglobin) in sample, RF positive factors, pregnant sample and AFP positive sample, the antibody positive sample of related diseases (HAV, HCV, hepatitis B, syphilis, rubella, VZV, etc.) will not interfere the detection result.
3. Do not use heat-inactivated samples, heat inactivation will lead the degradation of IgM in sample.
4. Samples can be stored at 2-8°C for one week. If samples not tested in a week, store them at below -15°C and avoid freeze-thaw cycles.
5. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

Assay procedure

Restore all reagents and samples to room temperature ($25\pm 2^{\circ}\text{C}$) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at $2-8^{\circ}\text{C}$.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 1 well for blank control, 3 wells for negative control and 1 wells for positive control. **Samples need test in duplicate** (Blank well is not necessary for dual-wavelength detection).
2. **Add sample:**
 - a) Add 100 μL of **Negative/Positive Control** respectively to 3 negative control wells, 1 positive control wells, keep the blank control well empty.
 - b) Add 100 μL of **Sample Diluent** to sample well, then add 10 μL of Serum sample.
3. **Incubate:** Gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with plate sealer. Incubate for 30 min at 37°C in shading light.
4. **Wash:** remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with **Wash Buffer** and immerse for 30-60 sec each time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP conjugate:** add 100 μL of **HRP Conjugate** to each well except the blank control well.
6. **Incubate:** cover the ELISA Microtiter plate with plate sealer. Incubate for 30 minutes at 37°C in shading light.
7. **Wash:** repeat step 4.
8. **Add substrate:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** to each well (Including the blank control well). Gently tap the plate to mix thoroughly. Cover with a new plate sealer. Incubate for 15 min at 37°C in shading light.
9. **Stop reaction:** add 50 μL of **Stop Solution** to each well (Including the blank control well), gently tap the plate to mix thoroughly.
10. **OD Measurement:** set the Microplate Reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not essential when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 30 min.**

Reference value

1. Result analysis

- 1.1 Use each test result independently. Determine the result according to the Cut Off value.
- 1.2 Calculate the Cut Off: $\text{Cut Off}(C.O) = 0.10 + \text{negative control(NC) average A value}$ (when NC average $A_{450} < 0.05$, calculate at 0.05; while NC average $A_{450} \geq 0.05$, calculate at the actual value).

2. Quality control

- 2.1 Blank well (just chromogenic agent and stop solution) absorbance ≤ 0.08 .
- 2.2 Positive control (PC) $A_{450} > 0.30$.
- 2.3 Negative control (NC) $A_{450} < 0.08$.

The experimental result is valid if quality control is valid.

3. Determination of results

Positive result: Sample absorbance \geq Cut Off.

Negative result: Sample absorbance $<$ Cut Off.

Interpretation of results

Negative result indicates there is no HSV-I-IgM antibody detected in samples, while positive result means the opposite.

Limitations

1. This test is only used as the qualitative detection of HSV-I-IgM in serum of human.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.