Technical Manual

Human Herpes Simplex Virus Type I (HSV-1) IgG ELISA Kit

- Catalogue Code: HDES0047
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect-ELISA as the method to detect the HSV Ⅰ-IgG antibody in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified HSV-Ⅰ antigen. Samples are added to the ELISA Microtiter plate wells, the HSV-Ⅰ antibody in the sample will combine with the pre-coated antigen to form antigen-antibody compound. Free components are washed away. The HRP conjugated Mouse-anti-human IgG monoclonal antibody is added to each well and react with the compound to form antigen-antibody- HRP Conjugate compound. The substrate reagent is added to initiate the color developing reaction. The presence of HSV-Ⅰ-IgG can be determined according to the absorbance value by using a microplate reader with 450 nm (630 nm) wavelength.

2. Kit components

Item	Specification
ELISA Microtiter plate	96 wells
HRP Conjugate	12 mL
Sample Diluent	12 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Positive Control	1 mL
Negative Control	1 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. Experimental instrument

- Microplate 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 water
- Absorbent paper

4. Sample preparation

1. **Serum**: Fresh collected serum specimens should be fully centrifugal, then take clear liquid for test. Suspended fibrous protein may cause a false positive result if not fully precipitated. Obviously contaminated samples can't be detected.
2. Anticoagulant (EDTA, sodium citrate and heparin) in samples do not affect the result of the experiment in general. Endogenous interference substances in serum such as blood lipids, cholerythrin, hemoglobin, rheumatoid factors positive samples, AFP positive samples and pregnant samples may not affect the results in general. Common positive samples of specific virus antibodies, such as HAV, HBV, HCV, TP, RV and related diseases, will not affect the results.
3. There should be no microorganism contained in the samples. Samples can be stored at 2~8° C for one week. If samples not tested in a week, store them at below -20° C and avoid freeze-thaw cycles.
4. **Wash Buffer**: The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.

5. Assay procedure

Restore all reagents and samples to room temperature (25°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° C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. Set 1 well for blank control, 1 wells for negative control and 3 well for positive control. **Samples need test in duplicate.**
2. **Add sample:**
   a) Add 100 μL of control serum respectively to 1 wells for negative control, 3 well for positive control, keep the blank control well empty. (Blank well is not necessary for dual-wavelength detection).
   b) Dilute the tested Serum with Sample Diluent at 1:10 into sample well (add 100 μL of Sample Diluent and add 10 μL of serum sample), mix fully.
3. **Incubate:** cover the ELISA Microtiter plate with 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 30-60s 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, mix fully.

6. **Incubate:** cover the ELISA Microtiter plate with 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. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. Incubate for 10 minutes at 37°C in shading light.
9. **Stop reaction:** add 50 μL of **Stop Solution** to each 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.**

6. Reference value

Normally, blank well (just **s**ubstrate agent and stop solution) absorbance: A450 ≤0.08; positive control (PC): A_{450} > 0.30 and average A value of negative control (NC): A_{450} < 0.08.

7. Interpretation of test results

Use each test result independently. Determine the result according to the Cut Off value.
Cut Off (C.0) = 0.10 + average A value of negative control(NC) (when average A_{450} of NC < 0.05, calculate at 0.05; while average A_{450} of NC ≥ 0.05, calculate at the actual value).

1. Positive result: average A value of sample ≥ Cut Off.
2. Negative result: average A value of sample < Cut Off.
3. Negative result indicates no HSV-Ⅰ-IgG antibody detected in samples, while positive result is means the opposite.

8. Limitations of test method

1. This test is only used as the qualitative detection of HSV-Ⅰ-IgG antibodies 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.

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