Technical Manual

Human Herpes Simplex Virus Type II (HSV-2) IgM ELISA Kit

- Catalogue Code: HDES0048
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Capture-ELISA as the method to detect the HSV-Ⅱ-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-Ⅱ antigen) is added to each well and incubate. The "HRP conjugated HSV-Ⅰ antigen" will react with the "anti-μ chain–HSV-Ⅱ-IgM antibody" compound to form "anti-μ chain- HSV-Ⅱ IgM antibody -HRP conjugated HSV-Ⅱ antigen" compound. The TMB substrate is added after washing to initiate the color developing reaction. The presence of HSV-Ⅱ-IgM antibody can be determined according to the OD value after colorimetric assay with the Micro plate Reader.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96T/96T*2
Positive Control	1 mL
Negative Control	1 mL
HRP Conjugate Working Solution	6 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Valve Bag	1 copy
Manual	1 copy

3. 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 water
- Absorbent paper
- Loading slot for Wash Buffer

4. Requirements of sample

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. Assay procedure

1. Bring all reagents to room temperature for 30 min. Dilute the 20× Concentrated Wash Buffer for 20 times with deionized or distilled water.
2. **Add sample:** Set 1 well for blank control, 3 wells for negative control and 1 well for positive control respectively. Add 50 μL of control serum or sample serum to each control well/ sample well. Keep the blank control well empty. Gently shake the plate to ensure thorough mixing.
3. **Incubate:** Cover the plate with the plate sealer. Incubate for 30 minutes at 37° C.
4. **Wash:** After incubation, 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 seconds each time.
5. **Add the HRP conjugate:** Add 50 μL of HRP Conjugate Working Solution to each well except the blank control well.
6. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 minutes at 37°C.
7. **Wash:** After incubation, 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 seconds each time.
8. **Add substrate:** Add 50 μL of Substrate A and 50 μL of Substrate B to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate for 15 minutes at 37°C in dark.
9. **Stop reaction:** Add 50 μL of Stop Solution to each well, gently tap the plate to ensure

thorough mixing.

10. **OD Measurement:** Set the Micro-plate Reader wavelength at 450 nm/630 nm to detect the A value of each well. Or set the Micro-plate Reader to zero with the blank well to determine the A value of each well directly when using single wavelength 450 nm for detection. (Note: read the A value within 10 minutes after the termination of the reaction.)

6. 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: Cut Off(C.0) = 0.10 + negative control(NC) average A value (when NC average A450＜0.05, calculate at 0.05; while NC average A450≥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≥Cut Off.
   Negative result: Sample absorbance＜Cut Off.

7. Interpretation of results

Negative result indicates there is no HSV-Ⅱ-IgM antibody detected in samples, while positive result means the opposite. The positive result of HSV-Ⅱ-IgM antibody is an important index of HSV-Ⅱ infection.

8. Limitations

1. All high sensitivity immune experiment system exists potential non-specificity. Therefore, unacceptable positive results may be caused by biological false positive of ELISA method.
2. Any positive result should be determined combined with clinical information.

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