Technical Manual

Human Epstein-Barr Virus (EBV) Viral Capsid Antigen (EB-VCA) IgM ELISA Kit

- Catalogue Code: HDES0060
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Capture-ELISA as the method to detect the EB-VCA-IgM antibody 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, the EB-VCA-IgM antibody in the sample will be captured. Free components are washed away (including the specific IgG antibody). The HRP conjugated EB-VCA antigen is added to each well, the EB-VCA-IgM antibody in captured IgM will specific bind to the HRP conjugated EB-VCA antigen. The substrate reagent is added to initiate the color developing reaction. The presence of EB-VCA-IgM 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 samples should be fully centrifuged, 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. Avoid of samples with hyperlipidemia (triglyceride≥20 g/L), hemolysis (hemoglobin ≥10 g/L) or jaundice (bilirubin ≥0.2 g/L).
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, 2 wells for negative control and 1 well for positive control. **Samples need test in duplicate.**
2. **Add sample:**
   a) Add 100 μL of control serum respectively to 2 wells for negative control, 1 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 15 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 10 min.**

6. Reference value

Normally, blank well (just **s**ubstrate agent and stop solution) absorbance: A450 ≤0.08; positive control (PC): A_{450} > 0.80 and average A value of negative control (NC): A_{450} < 0.1.

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 there is no EB-VCA-IgM antibody detected in samples, while positive result means the opposite.

8. Limitations of test method

1. This test is only used as the qualitative detection of EB-CA-IgM 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.
3. In the early stage of infection, IgM did not occur or has a low titer, and these situations will lead to negative results. It is recommended to remind the patients to recheck within 7-14 days. Make a parallel detection of the last sample to confirm whether there is seroconversion or titer elevation.

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