Technical Manual

Human Epstein-Barr Virus (EBV) Nuclear Antigen (EB-NA1) IgA ELISA Kit

- Catalogue Code: HDES0006
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect-ELISA as the principle to detect the Epstein-Barr Virus Nuclear Antigen (EB-NA1) IgA in human serum and plasma. The ELISA Microtiter plate provided in this kit has been pre-coated with purified EB- NA1 antigen. Samples are added to the ELISA Microtiter plate wells and the EB-NA1-IgA in which will combine with the pre-coated antigen to form antigen-antibody compound. Free components are washed away. The HRP conjugated Mouse-anti-human IgA antibody is added to each well and react with the compound to form “antigen- antibody-HRP antibody” compound. The TMB substrate is added to initiate the color developing reaction. The presence of EB-NA1-IgA can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Positive Control	1 mL
Negative Control	1 mL
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
Plate Sealer	3 pieces
Sealed Bag	1
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
- **Sample preparation**

- Human serum can be used as detected sample. Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. Suspended fibrous protein may cause a false positive.

- Avoid of samples with suspended fibrous protein, aggregation or severe hemolysis (hemoglobin ＞10 g/L), hyperlipemia (triglyceride ＞20 g/L), high bilirubin (bilirubin＞0.2 g/L). Obviously contaminated samples can't be detected.
- Samples can be stored at 2~8° C for one week. If samples not tested in a week, store them below --15° C no more than a month, and avoid freeze-thaw cycles.
- Bring all reagents to room temperature (18~25° C) for more than 30 min before use. Freezing samples should be mixed fully before test.

4. Assay procedure

Bring all reagents to room temperature for 30 min. Dilute the 20×Concentrated Wash Buffer for 20 times with distilled water.

1. **Add sample:**
   a) Take out Micro-plate and mark it, reserve 1 well for blank control (empty), 3 wells for negative control, 2 wells for positive control (100 µL control serum for each well). (Blank well is not necessary for dual-wavelength detection)
   b) Dilute the tested serum with Sample Diluent at 1:11 (add 100 µL of Sample Diluent to the reaction well, and then add 10 µL of sample), mix fully.
   c) Gently tap the plate to ensure thorough mixing.
2. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
3. **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 sec each time.
4. **HRP conjugate:** Add 100 µL of HRP Conjugate Working Solution to each well except the blank control well.
5. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
6. **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 sec each time.
7. **Add substrate:** Add 50 µL of Substrate Reagent A and 50 µL of Substrate Reagent B to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate for 15 min at 37° C in dark.
8. **Stop reaction:** Add 50 µL of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
9. **OD Measurement:** Set the Micro-plate 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 needed when using dual wavelength 450 nm/630 nm for detection.

5. Reference value

1. **Result analysis**
   (1) Use each test result independently. Determine the result according to the Cut Off value.
   (2) Calculate the Cut Off: Cut Off(C.0) = 0.10 + negative control (NC) average A value (when NC average A_{450} ＜ 0.05, calculate at 0.05; while NC average A_{450} ≥ 0.05, calculate at the actual value).
2. **Quality control**
   (1) Blank well (just chromogenic agent and Stop Solution) absorbance ≤ 0.08.
   (2) Positive control (PC) A_{450} ≥ 0.80.
   (3) Negative control (NC) A_{450} ＜ 0.10.
   The experimental result is valid if quality control is valid.
3. **Determination of results**
   (1) Positive result: Sample absorbance ≥ Cut Off.
   (2) Negative result: Sample absorbance ＜ Cut Off.

6. Interpretation of test results

Negative result indicates no EB- NA1-IgA antibody detected in samples, while positive result means the opposite.

7. Limitations of test method

The detection results of this kit are only for clinical reference. For confirmation of the diagnosis, please combine the clinical symptoms and other methods of detection, this detection cannot be used as the only criteria for clinical diagnosis.

8. Notes

1. 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.
2. 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.
3. The ELISA 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.
4. 20×Concentrated Wash Buffer at low temperature condition is easy to crystallize, it should be adjusted to room temperature to dissolve completely before use.
5. Each well must be filled with liquid when washing to prevent residual free enzyme.
6. The tested sample should be kept fresh.

7. The results shall depend on the readings of the Micro-plate Reader.
8. Do not use components from different batches of kit.

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