Technical Manual

Human Epstein-Barr Virus (EBV) Early Antigen (EB-EA) IgA ELISA Kit

- Catalogue Code: HDES0004
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect-ELISA as the principle to detect the Epstein-Barr Virus Early Antigen (EB-EA) IgA in human serum and plasma. The ELISA Microtiter plate provided in this kit has been pre-coated with purified EB-EA antigen. Samples are added to the ELISA Microtiter plate wells and the EB-EA-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-EA-IgA can be determined according to the OD value after colorimetric assay with the Microplate 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

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
- **Sample preparation**

- **Serum**: Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. Suspended fibrous protein may cause a false positive. Samples can be stored at

2~8° C for one week and stored at -20 ° C for more than a week. Avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.

- 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. Freezing samples should be mixed fully before test.
- **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.

4. Assay procedure

Restore all reagents and samples to room temperature 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. **Add sample:**
   a) Take out Microtiter plate and mark it, reserve 1 well for blank control (empty), 3 wells for negative control, 2 well for positive control. Add 100 µL of **negative control**/**positive control** to **negative control**/**positive control** wells, 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.
   c) Gently tap the plate to mix thoroughly.
2. **Incubate:** cover the ELISA Microtiter plate with plate sealer. Incubate for 30 minutes at 37°C in shading light.
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 30-60s 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).
4. **HRP conjugate:** Add 100 µL of **HRP Conjugate** to each well except the blank control well.
5. **Incubate:** cover the ELISA Microtiter plate with sealer. Incubate for 30 minutes at 37°C in shading light.
6. **Wash:** repeat step 3
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 mix thoroughly. Cover with a new plate sealer. Incubate for 15 minutes at 37°C in shading light.
8. **Stop reaction:** add 50 µL of **Stop Solution** to each well, gently tap the plate to mix

thoroughly.

9. **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.

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 $A_{450} \geqslant 0.05$, calculate at the actual value).
2. **Quality control**
   (1) Blank well (just chromogenic agent and Stop Solution) absorbance $\leqslant 0.08$.
   (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 $\geqslant$ Cut Off.
   (2) Negative result: Sample absorbance $<$ Cut Off.

6. Interpretation of test results

Negative result indicates no EB-EA-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 diagnosis.

8. Notes

1. This kit is for research use only. It is disposable and cannot be used repeatedly.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. 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.
4. 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.

5. 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.
6. 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.
7. The results shall depend on the readings of the micro-plate Reader.
8. Do not use components from different batches of kit.
9. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

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Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com