Technical Manual

Human Enterovirus 71 (EV71) IgG ELISA Kit

- Catalogue Code: HDES0069
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect-ELISA as the method to detect the EV71-IgG in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified Enterovirus 71 Virus antigen. Samples are added to the ELISA Microtiter plate wells and the EV71 antibody 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 IgG antibody is added to each well and react with the compound to form “EV71 antigen- EV71 antibody-HRP antibody” compound. The substrate reagent is added to initiate the color developing reaction. The presence of EV71-IgG can be determined by measuring the absorbance value by using a microplate reader with 450 nm (630 nm) wavelength.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
20×Concentrated Wash Buffer	50 mL
HRP Conjugate	12 mL
Sample Diluent	12 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 or distilled water
- Absorbent paper
- Stroke-physiological saline solution

4. Sample preparation

1. **Serum**: Human serum can be used as detected sample. Fresh collected serum samples should be fully centrifuged, then take clear liquid for assay. The suspended fibrous protein may cause a false positive result if not fully precipitated. Obviously contaminated samples can't be detected. 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.
2. **Diluted serum:** Dilute **Serum** with stroke-physiological saline solution at 1:10 (take 100 μL of stroke-physiological saline solution to a clean centrifuge tube, add 10 μL of serum and mix thoroughly).
3. Anticoagulants (heparin sodium, EDTA, sodium citrate) has no interference to the assay results. In general, endogenous interferent (such as blood fat, cholerythrin, hemoglobin) in sample, RF factor and related diseases (HEV, HCV, hepatitis B, EB virus, CMV, rubella, etc.) will not interfere the detection result.
4. **Wash Buffer**: The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, 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, 3 wells for negative control and 1 well for positive control. **Samples need test in duplicate.** (Blank well is not necessary for dual-wavelength detection)
2. **Add sample:**
   (1) Add 100 μL of control serum respectively to 3 negative control wells, 1 positive control well, keep the blank control well empty. (Blank well is not necessary for dual-wavelength detection)
   (2) Dilute the **Diluted serum** with **Sample Diluent** at 1:10 into sample well (add 100 μL of Sample Diluent and add 10 μL of **Diluted serum**), mix fully.
3. **Incubate:** Gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 50 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 30-60 sec 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.
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 min at 37° C in shading light.
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 Microplate 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. **Note: Read the results within 30 min.**

6. Reference value

Normally, blank well (just **s**ubstrate agent and stop solution) absorbance: OD ≤ 0.07; positive control (PC): OD > 0.60 and average OD value of negative control (NC): OD < 0.15

7. Interpretation of the results

Cut Off (C.0) = 0.10 + average OD value of negative control (NC) (When average OD value of NC < 0.07, calculate at 0.07; while average OD value of NC ≥ 0.07, calculate at the actual value).

(1) Positive result: OD value of sample ≥ Cut Off.
(2) Negative result: OD value of sample < Cut Off.
(3) Negative result indicates no EV71 -IgG 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 EV71-IgG 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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