Technical Manual

Human Adenovirus (ADV) IgA ELISA Kit

- Catalogue Code: HDES0017
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect-ELISA as the principle to detect the ADV-IgA in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified adenovirus antigen. Samples are added to the ELISA Microtiter plate wells and the adenovirus 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 IgA antibody is added to each well and react with the compound to form “adenovirus antigen-adenovirus antibody-HRP antibody” compound. The TMB substrate is added to initiate the color developing reaction. The presence of ADV-IgA can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

2. Kit components

Item	Specification
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

4. Requirements of sample

1. Serum can be used as detected sample. Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not fully precipitated. Avoid of samples with hyperlipidemia (triglyceride≥20 g/L), hemolysis (hemoglobin ≥10 g/L) or jaundice (bilirubin ≥0.2 g/L). Obviously contaminated samples can't be detected.
2. Do not use heated inactivated samples. Heat inactivation will degrade antibodies.
3. Samples can be stored at 2~8° C for one week. If samples not tested in a week, store them below -20° C and avoid freeze-thaw cycles.
4. Bring all reagents to room temperature (18~25° C) for more than 30 min before use. Freezing samples should be mixed fully before test.

5. 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:**
   (1) Take out Micro-plate and mark it, reserve 1 well for blank control (empty), 3 wells for negative control, 2 well for positive control (100 µL control serum for each well). (Blank well is not necessary for dual-wavelength detection)
   (2) 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) 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.

6. 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.

7. Interpretation of results

1. Negative result indicates there is no ADV-IgA antibody detected in samples, while positive result means the opposite.
2. The positive result of ADV-IgA antibody is an important index of ADV acute infection.

8. Limitations of test method

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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