Technical Manual

Human Adenovirus Type 7 (ADV-7) IgM ELISA Kit

- Catalogue Code: HDES0085
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Capture-ELISA as the principle to detect the Adenovirus Type 7 (ADV-7) IgM in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified ADV-7 antigen. Samples are added to the ELISA Microtiter plate wells and the ADV-7-IgM 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 IgM 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 ADV-7-IgM can be determined according to the OD value after colorimetric assay with the Microtiter 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

- Microtiter 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. Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. 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.
3. 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.
4. 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.
5. 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.
6. The results shall depend on the readings of the Microtiter plate Reader.
7. **Each reagent is optimized for use in the HDES0085. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0085 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

5. Storage and expiry date

Store at 2-8° C.Avoid freeze.

Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample preparation

1. **Serum**: Human serum can be used as detected sample. Fresh collected 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.
2. Avoid of samples with suspended fibrous protein, aggregation or severe hemolysis (hemoglobin>10g/L), hyperlipemia (triglyceride>20g/L), high bilirubin (bilirub>0.2g/L). Obviously contaminated samples can't be detected.
3. **Wash Buffe**r: 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.

6. 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. **Add sample:**
   a) Add 100 μL of control serum respectively to 3 negative control wells, 1 positive control well, keep the blank control well empty.
   b) Dilute the tested **Serum** with **Sample Diluent** at 1:10 into sample well (except blank/ negative/ positive well) (add 100 μL of **s**ample diluent and add 10 μL of Serum), mix fully.
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 Microtiter 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.

7. 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.

8. Interpretation of test results

Negative result indicates no ADV-7-IgM antibody detected in samples, while positive result means the opposite. ADV-7-IgM is the important indicator of ADV-7 acute infection.

9. Limitations of test method

1. This test is only used as the qualitative detection of ADV-7-IgM antibodies in serum of human.
2. 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.

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