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(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

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

Catalog No: E-HD-E091

96T/96T*2

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: <u>techsupport@elabscience.com</u>
Website: <u>www.vetassay-elab.com</u>

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This ELISA kit uses Indirect-ELISA as the principle to detect the Adenovirus Type 7 (ADV-7) IgG 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-IgG 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 "antigen- antibody-HRP antibody" compound. The TMB substrate is added to initiate the color developing reaction. The presence of ADV-7-IgG can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Positive Control	1 mL
Negative Control	1 mL
HRP Conjugated Working Solution	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

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

- 1. 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.
- 2. 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.
- 3. Samples can be stored at $2\sim8^{\circ}$ 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.

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 μ L control serum for each well). (Blank well is not necessary for dual-wavelength detection)
- b) Dilute the tested serum with Sample Diluent at 1:10 (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 μ L of Substrate Reagent A and 50 μ L 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 $^{\circ}$ 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.

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 $A_{450} \ge 0.05$, calculate at the actual value).

2. Quality control

- (1) Blank well (just chromogenic agent and Stop Solution) absorbance ≤ 0.08 .
- (2) Positive control (PC) $A_{450} \ge 0.80$.
- (3) Negative control (NC) $A_{450} < 0.1$.

The experimental result is valid if quality control is valid.

3. Determination of results

- (1) Positive result: Sample absorbance \geq Cut Off.
- (2) Negative result: Sample absorbance < Cut Off.

Interpretation of test results

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

Limitations of test method

The test results are for clinical reference only, Any positive results should be combined with clinical information to determine the result.

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.

Storage and shelf life

Store unopened at 2 to 8° C. Do not freeze.

Please store the opened kit at $2\sim8^{\circ}$ C, protect from light and moisture. The shelf life of the opened kit is up to 1 months.

Expiry date: expiration date is on the box.