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Human Echovirus (ECHO) IgG ELISA Kit

Catalog No: E-HD-E015

96T/96T*2

Version Number:	V1.2
Replace version:	V1.1
Revision Date:	2025.03.14

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

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

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Email: techsupport@elabscience.com

Website: www.vetassay-elab.com

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 Echovirus (ECHO) IgG in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified ECHO antigen. Samples are added to the ELISA Microtiter plate wells and the ECHO 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 "antigen-antibody-HRP antibody" compound. The TMB substrate is added to initiate the color developing reaction. The presence of ECHO-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 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

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℃ Incubator or water bath

Deionized water

Absorbent paper

Loading slot for Wash Buffer

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℃. Do not freeze.

Please store the opened kit at 2~8℃, 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.

Sample preparation

1. Fresh collected serum specimens should be fully centrifugal, then take clear liquid for test. The suspended fibrous protein may cause a false positive result if not fully precipitated.
2. Samples can be stored at 2~8℃ for one week. If samples not tested in a week, store them below -20℃ 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 μL 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 serum 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℃.
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℃.
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℃ 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. **Note: Read the results within 10 min.**

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} ＜ 0.1.
   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.

Interpretation of test results

1. Negative result indicates no ECHO-IgG antibody detected in samples, while positive result is just the opposite.
2. The positive result of ECHO-IgG antibody is an important index of ECHO acute infection.

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 combined with clinical information to determine the result.