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Lieferung & Zahlungsart

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Zuschläge

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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8th Edition, revised in February, 2018

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

Human Coxsackievirus A16 (CV-A16) IgM ELISA Kit

Catalog No: E-HD-E139

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: 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 Capture-ELISA as the method to detect the CA16-IgM in the sample. The micro-plate is pre-coated with Mouse-anti-human IgM (μ chain). CA16-IgM in samples will be captured after adding the samples to wells. After washing completely, add CA16 antigen conjugated Horseradish Peroxidase (HRP), and it will react with the "anti-μ chain CA16 IgM" compound to form "anti-μ chain- CA16 IgM-HRP conjugated CA16 antigen". Wash and remove the free components. Add the TMB substrate to initiate color developing reaction. The presence of CA16-IgM antibody 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
HRP Conjugate	12 mL
20×Concentrated Wash Buffer	50 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 copy
Manual	1

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℃ Incubator or water bath

Deionized water

Absorbent paper

Loading slot for Wash Buffer

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 micro-plate Reader.
7. **Each reagent is optimized for use in the E-HD-E139. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E139 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

Storage and shelf life

Store unopened at 2-8℃. 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.

Requirements of sample

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℃ for one week and stored at -20℃ for more than a week. Avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.
2. Anticoagulant (such as EDTA, sodium citrate and heparin, etc.) in samples do not affect the results.
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.

Assay procedure

Restore all reagents and samples to room temperature (25℃) 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℃.

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 (Blank well is not necessary for dual-wavelength detection). **Samples need test in duplicate.**

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.

(2) Dilute the tested **Serum** with **Sample Diluent** at 1:10 into sample well (except blank/negative /positive well) (add 100 μL of sample diluent and add 10 μL of sample), mix fully.

3. **Incubate:** Cover the ELISA Microtiter plate with sealer. Incubate for 30 min at 37℃.

4. **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.

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 min at 37℃.

7. **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.

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 ensure thorough mixing. Cover with a new plate sealer. Incubate for 15 min at 37℃ in dark.

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 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} ≥ 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.

Interpretation of test results

1. Negative result indicates no CA16-IgM antibody detected in samples, while positive result means the opposite.
2. The concentration of CA16-IgM may be very low during the early stage of infection, it may cause negative result. The patient should review the CA16-IgM level in 7~14 days.
3. The reference value of the serological antibody testing for patients with immunity damage or accepted immunosuppressive therapy is limited.
4. The positive result of CA16-IgM may appear during the primary infection, as well as previous infection.

Limitations of test method

1. This test is only used as the qualitative detection of CA16-IgM antibodies in serum and plasma 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.