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Human Echovirus (ECHO) IgM ELISA Kit

Catalog No: GEH126

96T

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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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 ECHO-IgM in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with Mouse-anti-human IgM (μ chain). Samples are added to the ELISA Microtiter plate wells and the IgM antibody in which will be captured. Free components are washed away (including the specific IgG antibody). HRP conjugated ECHO antigen is added to each well and react with the compound to form "Mouse-anti-human IgM (μ chain) - ECHO IgM - ECHO antigen- HRP conjugated anti-ECHO antibody" compound. The TMB substrate is added after washing to initiate the color developing reaction. The presence of ECHO-IgM can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

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 piece
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

Requirements of sample

1. Human 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. Obviously contaminated samples can't be used.
2. The samples with hyperlipidemia (triglyceride≥20 g/L), hemolysis (hemoglobin ≥10 g/L) or jaundice (bilirubin ≥0.2 g/L) can't be detected.
3. Samples can be stored at 2~8℃ for one week or stored at -20 ℃ for more than a week. Avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.

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) Reserve 3 wells for negative control, 2 wells for positive control (100 μL of corresponding control solution for each well). Set 1 well for blank control (empty) (Blank well is not necessary for dual-wavelength detection)
   (2) 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.
   (3) Gently tap the plate to mix thoroughly.
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 mix thoroughly. 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 mix thoroughly.
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 + A value of average negative control (NC) (when A450 of average NC ＜ 0.05, calculate at 0.05; while A450 of average NC ≥ 0.05, calculate at the actual value).
2. **Quality control**
   (1) Blank well (just chromogenic agent and stop solution): A450 ≤ 0.08.
   (2) Positive control (PC): A450 ＞ 0.80.
   (3) Negative control (NC): A450 ＜ 0.10.
   The experimental result is valid if quality control is valid.
3. **Determination of results**
   (1) Positive result: A450 of Sample ≥ Cut Off.
   (2) Negative result: A450 of Sample ＜ Cut Off.

Interpretation of results

1. Negative result indicates there is no ECHO-IgM antibody detected in samples, while positive result means the opposite.
2. The positive result of ECHO-IgM 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. In the early stage of infection, IgM did not occur or has a low titer, and these situations will lead to negative results. It is recommended to remind the patients to recheck within 7-14 days. Make a parallel detection of the last sample to confirm whether there is seroconversion or titer elevation.
3. The reference value of serum antibody detection of patients with impaired immune function or patients receiving immunosuppressive therapy is limited.
4. Any positive result should be determined combined with clinical information.

Notes

1. This kit is for research use only. It is disposable and cannot be used repeatedly.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. 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.
4. 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.
5. 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.
6. 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.
7. The results shall depend on the readings of the micro-plate Reader.
8. Do not use components from different batches of kit.
9. 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 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.