Technical Manual

Human Hantavirus (HV) IgM ELISA Kit

- Catalogue Code: HDES0015
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Capture-ELISA as the method to detect the HV-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). The HRP conjugated HV antigen is added to each well, the HV-IgM antibody will bind to the HRP conjugated HV antigen. Free components are washed away and the TMB substrate is added to initiate the color developing reaction. The presence of HV-IgM can be determined according to the OD value after colorimetric assay with the Micro-plate Reader.

2. 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

3. Other materials required but not supplied

- 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

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 micro-plate Reader.
7. **Each reagent is optimized for use in the HDES0015. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0015 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 unopened at 2-8° C. Do not freeze.
Please store the opened kit at 2-8° 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 packing box.

6. Sample preparation

1. **Serum:** 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 detected. 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 hyperlipidemia (triglyceride > 20 g/L), hemolysis (hemoglobin > 10 g/L) or jaundice (bilirubin > 0.2 g/L).
3. Do not use heat inactivated or samples containing microorganisms, heat inactivation will degrade IgG antibodies in the sample.
4. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

7. 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. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 1 well for blank control, 3 wells for negative control and 2 wells for positive control. **Samples need test in duplicate** (Blank well is not necessary for dual-wavelength detection).
2. **Add sample:**
   a) Add 100 µL of **Positive/Negative Control** respectively to **Positive/Negative Control** wells, keep the blank control well empty.
   b) Dilute the tested **Serum** with **Sample Diluent** at 1:10 into sample well (add 100 µL of **Sample Diluent** and add 10 µL of **Serum** sample), mix fully.
3. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 30 min at 37° C in shading light.
4. **Wash:** 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. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP conjugate:** add 100 µL of **HRP Conjugate** to each well except the blank control well.
6. **Incubate:** cover the ELISA plate with sealer. Incubate for 30 min at 37° C in shading light.
7. **Wash:** repeat step 4.
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 mix thoroughly. Cover with a new plate sealer. Incubate for 15 min at 37° C in shading light.
9. **Stop reaction:** add 50 µL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
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 essential when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 10 min.**

8. Reference value

Blank well (just chromogenic agent and stop solution) absorbance: A450 ≤ 0.08, Positive control (PC) A_{450} > 0.80, Negative control (NC) A_{450} < 0.10.

9. Interpretation of the results

Cut Off(C.0) = 0.10 + average A value of negative control (NC) (when average A450 of NC < 0.05, calculate at 0.05; while average A450 of NC ≥ 0.05, calculate at the actual value).

(1) Positive result: Sample absorbance ≥ Cut Off.
(2) Negative result: Sample absorbance < Cut Off.
(3) Negative result indicates there is no HV-IgM antibody detected in samples, while positive result means the opposite.
(4) The positive result of HV-IgM antibody is an important index of HV acute infection.

10. Limitations of test method

1. This test is only used as the qualitative detection of HV- IgM in serum 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.

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