Technical Manual

Human Influenza A Virus (AIV) IgM ELISA Kit

- Catalogue Code: HDES0050
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Capture-ELISA as the method to detect the AIV-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). Then add the HRP conjugated AIV antigen to each well and incubate. The “HRP conjucated AIV antigen” will react with the “anti-μ chain-AIV IgM antibody” compound and form the “anti-μ chain-AIV IgM antibody -HRP conjugated AIV antigen” compound. The substrate reagent is added after washing to initiate the color developing reaction. The presence of AIV IgM antibody can be determined by measuring the absorbance value by using a microplate reader with 450 nm (630 nm) wavelength.

2. Kit components

Item	Specification
ELISA Microtiter plate	96 wells
20×Concentrated Wash Buffer	50 mL
HRP Conjugate	12 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 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. Other materials required but not supplied

- Microplate 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

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 microplate reader.
7. **Each reagent is optimized for use in the HDES0050. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0050 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 the kit at 2-8° C. Do not freeze any test kit components.
Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8° C.
Expiry date: expiration date is on the packing box.

6. Sample preparation

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.
2. Anticoagulant (EDTA, sodium citrate and heparin sodium) in samples do not affect the result of the experiment in general. 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). Endogenous interference substances in serum such as rheumatoid factors positive samples, pregnant AFP positive samples may not affect the results.
3. 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.

4. Do not use heat inactivated specimens, heat inactivation will degrade IgM antibodies in the sample
5. **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:**
   (1) Add 100 μL of control serum respectively to 3 negative control wells, 2 positive control wells, keep the blank control well empty.
   (2) 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:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter 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 ensure thorough mixing. 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 ensure thorough mixing.
10. **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 essential when using dual wavelength 450 nm/630 nm for detection.

8. Reference value

Normally, blank well (just **s**ubstrate agent and stop solution) absorbance: A450 ≤ 0.08; positive control (PC): A_{450} > 0.80 and average A value of negative control (NC): A_{450} < 0.10.

9. Interpretation of results

Cut Off = 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: average A450 of Sample ≥ Cut Off.

(2) Negative result: average A450 of Sample < Cut Off.

(3) Negative result indicates no AIV-IgM antibody detected in samples, while positive result means the opposite.

10. Limitations of test method

1. This test is only used as the qualitative detection of AIV-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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