Technical Manual

Human Insulin (INS) IgG ELISA Kit

- **Catalogue Code: HDES0073**
- **Antibody ELISA Kit**
- **Research Use Only**

1. Test principle

This ELISA kit uses Indirect-ELISA as the method to detect the Insulin (INS) IgG antibody in human serum. The micro ELISA plate provided in this kit has been pre-coated with purified recombinant INS antigen. Samples are added to the ELISA Microtiter plate wells and the INS-Ab in which will combine with the pre-coated antigen to form antigen-antibody compound. Free components are washed away. The HRP conjugate is added to each well and react with the compound to form antigen- antibody-HRP conjugate compound. The substrate reagent is added to initiate the color developing reaction. The presence of INS-Ab can be determined according to the OD value after colorimetric assay with the Micro-plate reader.

2. 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 piece
Manual	1 copy

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

3. 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° C Incubator or water bath
- Deionized water
- Absorbent paper

4. 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. Suspended fibrous protein may cause a false positive. Obviously contaminated samples can't be detected.
2. Anticoagulant (EDTA, sodium citrate and heparin sodium) in samples do not affect the result of the experiment in general. Endogenous interfering substances in the serum such as blood fat and hemoglobin may cause false results.
3. There should be no microorganism contained in the samples. Samples can be stored at 2~8° C for one week. If samples not tested in a week, store them at below -20° C and avoid freeze-thaw cycles.
4. **Wash Buffer**: 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.

5. 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 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. **Samples need test in duplicate.**
2. **Add sample:**
   a) Add 100 µL of control serum respectively to 3 negative control wells, 1 positive control well, keep the blank control well empty. (Blank well is not necessary for dual-wavelength detection)
   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 sample), mix fully.
3. **Incubate:** cover the ELISA Microtiter plate with plate sealer. Incubate for 30 min at 37° C.
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, mix fully**.**
6. **Incubate:** cover the ELISA Microtiter plate with sealer. Incubate for 30 minutes 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 30 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 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 30 min.**

6. Reference value

Normally, blank well (just **s**ubstrate agent and stop solution) absorbance: A450 ≤0.08; positive control (PC): A_{450} >0.50 and average A value of negative control (NC): A_{450} <0.08.

7. Interpretation of test 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: average A value of sample ≥ Cut Off.
2. Negative result: average A value of sample < Cut Off.

8. Limitations of test method

1. This test is only used as the qualitative detection of INS- IgG-Ab 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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