Technical Manual

Human Anti-Insulin Antibody ELISA Kit

- Catalogue Code: HDES0116
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This reagent kit pre encapsulates recombinant human insulin in a reaction plate to capture insulin antibodies in the sample, washes out irrelevant substances, and then adds horseradish peroxidase (HRP) labeled mouse anti human IgG antibody for binding; Finally, a solid-phase antigen insulin antibody enzyme labeled anti human IgG complex is formed, which can be colored by the TMB colorimetric system to determine the presence of insulin antibodies in the human serum or plasma to be tested in the sample.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
HRP Conjugate	6 mL*2
Sample Diluent	6 mL*2
Negative Control	1 mL
Positive Control	1 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
20×Concentrated Wash	15 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 or distilled 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 micro-plate Reader.
7. **Each reagent is optimized for use in the HDES0116. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0116 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 at 2-8° C. Avoid freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.
Expiry date: expiration date is on the packing box.

6. Sample preparation

1. **Serum** samples are collected intravenously using conventional methods, while **Plasma** samples can be anticoagulated with conventional doses of heparin or sodium citrate.
2. Specimens measured within 3 days can be stored at (2-8) ° C- Store below 15 ° C for 3 months.
3. The specimen should avoid hemolysis and repeated freeze-thaw cycles.
4. Samples that are turbid or have sediment should be centrifuged or filtered to clarify before testing.
5. During the collection and storage process of serum that needs to be preserved, attention should be paid to aseptic operation.
6. **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.

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. Each experiment should have 3 negative control wells, 2 positive control wells, and 1 blank control well (the dual wavelength measurement mode may not have a blank control well).
2. Add 100 μL of **Sample Diluent** in order to each reaction well (excluding positive and negative controls), and do not add blank wells. Add 10 μL of the specimen to be tested and 100 μL each of the **Negative Control** and **Positive Control** to the corresponding wells.
3. Cover the reaction plate with a sealing film and place it in a 37 ° C incubator or water bath for 60 minutes of reaction.
4. 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. Add 100 μL of **HRP Conjugate** to each well, mix thoroughly, apply a sealing plate membrane, and incubate at 37° C for 30 min.
6. Repeat step 4 for washing.
7. Add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** to each well (Including blank control well). Gently tap the plate to mix thoroughly. Cover with a new plate sealer. incubate at 37° C for 15 min in shading light.
8. Add 50 μL of **Stop Solution** to each well (Including blank control well), gently tap the plate to mix thoroughly.
9. **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.

8. Result analysis

Cutoff value=mean OD value of negative control × 2.1
Positive: Sample OD value ≥ Cutoff value
Negative: Sample OD value < Cutoff value
Attention: ① The OD value of negative control quality control should be ≤ 0.1 (if there is one negative control OD value greater than 0.1, it should be discarded. If there are two or more negative control OD values greater than 0.1, the experiment should be repeated. When the average OD value of three negative controls is less than 0.05, it should be calculated as 0.05). ② The OD value of positive control quality control should be ≥ 0.45 (if there is 1 well of positive control OD value less than 0.45, it should be discarded, and if the OD values of 2 wells of positive control are both less than 0.45, the experiment should be repeated).

9. Limitations of test method

This kit can only be used for qualitative testing.

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