# Technical Manual

# Human Insulin ELISA Kit

- **Catalogue Code: HDES0114**
- **Antibody ELISA Kit**
- **Research Use Only**

## 1. Test principle

Enzyme linked immunosorbent assay (ELISA) sandwich method was used to detect the content of human insulin in serum. Firstly, a monoclonal antibody is coated onto a microplate to prepare a solid-phase antibody. Then, the serum to be tested and another monoclonal antibody labeled with horseradish peroxidase are added to form a complex of coated antibody insulin labeled antibody. After color development, the absorbance value (OD value) is measured using an enzyme-linked immunosorbent assay (ELISA) reader. The concentration absorbance curve is fitted using a computer or graph to calculate the human insulin content in the serum to be tested.

## 2. Kit components

| Item | Specifications |
|---|---|
| ELISA Microtiter plate | 96 wells |
| Standard Liquid | 0.5 mL each (0, 10, 20, 40, 80, 160 mIU/L) |
| HRP Conjugate | 6 mL |
| Quality Control Solution | 1 mL*2<br>Level 1 is (11.6 ~19.2 mIU/L), Level 2 is (54.4 ~90.6 mIU/L) |
| 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 HDES0114. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0114 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. Collect the patient's fasting blood and postprandial blood 1 hour after eating, collect the blood into a test tube, and record the collection time.
2. Separate the **Serum** from the blood clot as early as possible (centrifuge at 3000rpm for more than 6 minutes) to avoid hemolysis. Hemolytic samples can affect the accuracy of test results.
3. The sample is stored in a sealed container at 2-8 ° C and can be stored for 3 months below -15 ° C to avoid repeated freezing and thawing.
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.

## 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. Remove the pre-packaged board from the sealed bag, set a blank control hole, and do not add any liquid; Two holes are set up for each calibration point, and 50 μL of **Standard Liquid** is added to each hole; Add 50 μL of **Quality Control Solution** or test **Serum** directly to each of the remaining detection wells. Then add 50 μL of **HRP Conjugate** to each well, mix thoroughly, apply a sealing plate membrane, and incubate at 37° C for 1 hour.
2. Remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 3 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).
3. 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 at 37° C for 15 min in shading light.
4. Add 50 μL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
5. **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

1. The dual wavelength enzyme-linked immunosorbent assay (ELISA) reader does not require a blank control well or zero point adjustment. A single wavelength enzyme-linked immunosorbent assay (ELISA) reader must be equipped with a blank control well. First, zero the blank control well, and then measure.

2. Plotting method: Take the absorbance values of **Standard Liquid** S1~S5 as the vertical axis (log logarithmic coordinate) and the corresponding concentrations as the horizontal axis (log logarithmic coordinate), draw the calibration curve on a logarithmic coordinate paper, and find the content of the specimen to be tested on the calibration curve.

3. Computer: The concentration is calculated by the computer.

## 9. Limitations of test method

1. Severe hemolysis and chyle blood may affect the test results.

2. The sample tested for this product with a concentration of 3200μIU/mL did not exhibit a "hook effect".

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