Technical Manual

Human Carbohydrate Antigen 125 ELISA Kit

- Catalogue Code: HDES0110
- Antibody ELISA Kit
- Research Use Only

1. Test principle

The content of CA125 in serum was determined by enzyme-linked immunosandwich method and avidin and biotin system. Firstly, avidin was coated with microplates to prepare the fixation enzyme, and then biotin-labeled antibodies and calibrators or serum to be tested were added. After incubation and washing, horseradish peroxide-labeled mab was added to form the complex of avidin-biotin-labeled MAB -[CA125-enzyme-labeled mab]. After color development, OD value was determined by enzyme-labeled MAB. The concentration - absorbance curve was fitted by computer or drawing, and the content of CA125 in serum was calculated inversely.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (0，15，40，100，250U/mL)
HRP Conjugate	6 mL
Labeled Antibody	6 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 HDES0110. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0110 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** was quickly separated after blood collection to avoid hemolysis. Serum samples can be stored at 2~8° C for a week, long-term storage should be packaged, sealed and frozen below -15° C, can be stored for three months, and avoid repeated freezing and thawing (no more than 5 times).
2. **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. Before each experiment, the **Labeled Antibody** was taken according to the amount of experimental detection, according to the volume of 1/10 of the **HRP Conjugate**, and mixed with the enzyme binding to make the **enzyme conjugate mixture**. For example, take 3ml

of **HRP Conjugate**, add 0.3ml of **Labeled Antibody** and mix to make a mixture.

2. Take the pre-packaged sheet out of the sealed bag, set two wells for each calibrator and quality control product, and add the **Standard Liquid** 50 μL to each well; Add 50 μL of **serum** directly to each test well. Then each well (except blank control well) was added with **enzyme conjugate mixture** of 50 μL, thoroughly mixed, and affixed with sealing plate film. Incubate at 37° C for 60 min.
3. 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).
4. 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.
5. Add 50 μL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
6. **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

Using the linear fitting function, the concentration of each calibration product is logarithm (Log(concentration)) as X, the corresponding absorption value is logarithm (Log(OD)) as Y, and the double-logarithm (or full logarithm) log-log fitting equation is selected: Log(OD) = B Log(concentration) + A, and the concentration of the serum to be measured is calculated from the fitting line.

9. Limitations of test method

1. Rheumatoid factors, anti-animal antibodies and other factors that affect the immune response will affect the detection.
2. Anticoagulants, <4g/L hemoglobin, <160mg/L bilirubin, <10g/L cholesterol and <8.2mmol/L triglyceride had no significant effect on the determination results.
3. Hook effect: The maximum concentration of this kit without Hook effect is 4000U/mL. It is recommended that samples that exceed the range of the standard curve (250 U/mL) should be diluted with normal saline for re-determination, and the dilution ratio should not exceed 1:1000.

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