Technical Manual

Human Carcinoembryonic Antigen ELISA Kit

- Catalogue Code: HDES0106
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This reagent kit adopts the double antibody sandwich method. Using purified anti CEA monoclonal antibody coated microplates, CEA standards or samples, and enzyme labeled anti CEA monoclonal antibodies for reaction, a solid-phase antibody antigen enzyme labeled antibody complex is specifically formed. After substrate coloration, the concentration of CEA in the sample is proportional to the depth of coloration. Use an enzyme-linked immunosorbent assay (ELISA) reader to measure the OD values of each well. Calculate the content of the sample based on the standard curve.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (0,5,10,20,40,80 ng/mL)
HRP Conjugate	7 mL
Quality Control Solution	1 mL*2 Level 1 is (5.25~9.75 ng/mL), Level 2 is (17.5~32.5 ng/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 HDES0106. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0106 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** specimens are collected intravenously by routine method, and **Plasma** specimens can be anticoagulant with routine amounts of heparin or sodium citrate.
2. Specimens measured within 2 days can be stored at 4° C. Long-term storage should be frozen below -20° C.
3. Specimens should avoid hemolysis and repeated freeze-thaw.
4. Cloudy or precipitated specimens should be centrifuged or filtered to clarify and then tested.
5. Attention should be paid to aseptic operation during the collection and preservation of serum that needs to be preserved.
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. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.** Set 1 well for blank control.
2. **Add sample:** add 50 μL of **Standard Liquid** per well (Set two holes for each calibration point in sequence), then add 50 μL of **Quality Control Solution** or **test serum** directly to each of the remaining detection wells. Then add 50μL of **HRP Conjugate** to each well, mix thoroughly, apply a sealing plate membrane, and incubate at 37 ° C for 30 min.
3. **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).
4. **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 15 min at room temperature.
5. **Stop reaction:** 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

1. The OD value of each hole must be subtracted from the OD value of the zero standard (0 ng/mL).

2. Mapping method: Using double logarithmic coordinate paper, with the concentration of S1-S5 standard product as the horizontal coordinate and the corresponding OD value as the vertical coordinate, draw a standard curve, and find out the corresponding concentration according to the OD value of the sample.

3. Computer: Take the logarithm of the concentration of each S1-S5 standard product as X, and the logarithm of the corresponding OD value as Y, and use the least square method to fit the linear regression equation of one variable, and substitute the OD value of the sample into the equation to calculate the sample concentration. The equation is log[absorption value]=Blog[concentration]+A

4. Quality control: According to the sample method to determine the concentration of the quality control product, the measured value within the given range indicates that the experiment is

qualified.

9. Limitations of test method

Samples outside the range of the standard curve can not be directly calculated, and need to be diluted in sequence 1:10 after multiple concentrations are measured again. Finally, the measured CEA content multiplied by dilution ratio is the actual CEA content of the sample.

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