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CALBIOTECH

CA125 ELISA

Catalog No. CA239T (96 Tests)

INTENDED USE

The Calbiotech CA125 ELISA Kit is intended for the quantitative determination of the Cancer Antigen CA125 concentration in human serum. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

CA125, also known as MUC16, is a high molecular weight mucin-type glycoprotein. Research uses include mucin biology, glycosylation pattern studies, and evaluation of CA125 as a molecular component in panels for reproductive tract and epithelial cell biology research.

PRINCIPLE OF THE TEST

The CA125 ELISA test is an adapted solid phase 1-step sandwich ELISA. Samples, a biotinylated mouse anti-CA-125 capture antibody, and mouse anti-CA-125-HRP conjugate are all added to wells coated with streptavidin. CA-125 in the patient sample binds to the biotinylated capture antibody. The biotinylated capture antibody simultaneously binds to the streptavidin coated plate. Anti-CA-125–HRP enzyme conjugate forms a sandwich around captured CA-125. Unbound antibodies are washed off. TMB substrate is added resulting in the development of a blue color. The concentration of CA-125 is directly proportional to the color intensity developed. A standard curve is generated relating color intensity to CA-125 concentration.

MATERIALS PROVIDED		96 TESTS
1.	Microwells coated Streptavidin	12x8x1
2.	CA125 reference standards: 6 vials (ready to use)	0.5 mL
3.	CA125 Controls: 2 vials (ready to use)	0.5 mL
4.	Enzyme Conjugate Reagent	12 mL
5.	TMB Solution: 1 bottle (ready to use)	12 mL
6.	Stop Solution: 1 bottle (ready to use)	12 mL
7.	Wash Concentrate 20x: 1 Bottle	25 mL

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND PREPARATION

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze–thaw cycles.

REAGENT PREPARATION

1. Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25 °C).

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25 °C) and gently mix.

1. Secure the desired number of coated wells in the holder. Dispense 50µl of CA125 standards, specimens, and controls into the appropriate wells.
2. Dispense 100µl Enzyme Conjugate Reagent into each well.
3. Mix gently for 30 seconds. It is very important to have complete mixing in this setup.
4. Incubate for 60 minutes.
5. Remove the incubation mixture by emptying the plate content into a waste container.
6. Remove liquid from all wells. Wash wells three times with 300 µL of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual liquid droplets.
8. Dispense 100µl of TMB Reagent into each well. Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 15 minutes.
9. Stop the reaction by adding 50µl of Stop Solution to each well.
10. Read the absorbance at 450nm (using a reference wavelength of 630nm) with a microtiter plate absorbance reader within 15 minutes.

CALCULATION RESULTS

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CA125 in U/ml from the standard curve.

Example of Standard Curve

Results of a typical standard run with OD readings at 450nm shown in the Y axis against CA125 concentrations shown in the X axis. This standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

CA125 Values (U/ml)	Absorbance (450nm)
0	0.010
15	0.105
50	0.347
100	0.703
200	1.411
400	2.437

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.