CANCER ANTIGEN CA125 ENZYME IMMUNOASSAY TEST KIT
Catalog Number: OKBA00006

Aviva Systems Biology
10211 Pacific Mesa Blvd, Ste 401
San Diego, CA 92121

Enzyme Immunoassay for the Quantitative Determination of Ovarian Cancer Antigen CA125 in Human Serum

FOR IN Research Purposes Only

NOT FOR USE IN DIAGNOSTIC PROCEDURES

PRINCIPLE OF THE TEST

The CA125 ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.[iii] The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA125 molecule is used for solid phase immobilization (on the microtiter wells). A rabbit anti-CA125 antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CA125 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation at 37°C for 90 minutes, the wells are washed with Wash Buffer to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of CA125 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- *Antibody-Coated Wells (1 plate, 96 wells)*
  Microtiter Wells coated with CA125 MoAb
- *Reference Standard Set (1.0 ml/vial)*
  Contains 0, 15, 50, 100, 200, and 400 Unit/ml of CA125 in bovine serum with preservatives; liquid, ready to use
- *CA125 Enzyme Conjugate Reagent (13 ml)*
  Contains CA125 MoAb conjugated to horseradish peroxidase with preservatives
- *Wash Buffer Concentrate (20x) (50 ml)*
  Potassium phosphate buffer with tween 20
- *TMB Reagent (11 ml)*
  Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution
- *Stop Solution -1N HCl (11 ml)*
  Diluted hydrochloric acid

Materials required but not provided:

- Precision pipettes and tips: 100 µl
- Disposable pipette tips.
- Distilled water.
- Vortex mixer.
- Absorbent paper or paper towel.
- Microtiter plate reader.
- Graph paper.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 100 µl of CA125 standards, specimens, and controls into the appropriate wells.
2. Dispense 100 µl Enzyme Conjugate Reagent into each well.
3. Mix gently for 30 seconds. It is very important to have a complete mixing in this setup.
4. Incubate at 37°C for 90 minutes.
5. Remove the incubation mixture by emptying the plate content into a waste container.
6. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X).
7. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 20 minutes.
9. Stop the reaction by adding 100 µl of Stop Solution to each well.
10. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
11. Read the optical density at 450 nm with a microtiter plate reader ***within 15 minutes***.

CALCULATION OF 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.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density 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 (450 nm)
0	0.071
15	0.205
50	0.551
100	0.936
200	1.746
400	2.824