DRG® CA 125 (Ovarian Cancer) Antigen (EIA-1472)

Revised 24 Jan. 2011 rm (Vers. 3.1) **USA: RUO**

This kit is intended for Research Use 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. 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

1. Murine Monoclonal anti-CA125 **coated microtiter plate** with 96 wells.
2. **Enzyme Conjugate** Reagent, 13 ml.
3. CA125 reference **standards** containing; 0, 15, 50, 100, 200, and 400 Unit/ml of CA125, 1 ml each, ready to use.
4. **Wash Buffer Concentrate** (20X) 50 ml
5. **TMB Reagent** (One-Step), 11 ml
6. **Stop Solution** (1N HCl), 11 ml.

Materials required but not provided

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

Storage of Test Kit and Instrumentation

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air.
Opened test kits will remain stable until the expiration date shown, provided it is stored as described above.
A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

Reagent Preparation

1. All reagents should be brought to room temperature (18-25°C) before use.

2. To prepare **Wash Buffer (1X):** Add 50ml of Wash Buffer (20X) to 950 m of DI water. The diluted Wash Buffer is stable at 2-8ºC for 30 days. Mix well before use. Note" Any crystals that may be present due to high salt concentration must be redissolved at room temperature before making the dilution.

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)**.
   (Please do not use tap water.)
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

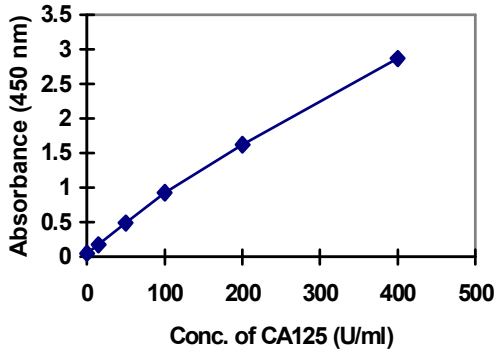
1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml on linear graph paper, with absorbance on the vertical (y) 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.

Example of Standard Curve

Results of a typical standard run with optical density readings at 450 nm 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**



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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 price.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

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