ErbaLisa® CA 125

Catalog No. IME00040 (96 Tests)

INTENDED USE
The ERBAlisa CA 125 ELISA kit is intended for the quantitative determination of the Cancer Antigen CA125 concentration in human serum.

CLINICAL UTILITY
Cancer Antigen 125 (CA125) is a surface antigen associated with epithelial ovarian cancer. In serum, CA125 is associated with a high molecular weight glycoprotein. Published studies have indicated that elevated serum CA125 levels can be found in individuals with serious endometroid, clear-cell and undifferentiated ovarian carcinoma. The serum CA125 concentration is greater than 35 units per ml in 60% of women with ovarian cancer and >80% of patients with disseminated ovarian cancer. The serum CA125 is elevated in 1% of normal healthy women, 3% of normal healthy women with benign ovarian diseases, 6% of patients with non-neoplastic conditions (including but not limited to first trimester pregnancy, menstruation, endometriosis, uterine fibrosis, acute salphingitis, hepatic diseases and inflammation of peritoneum, pericardium or pleura). Serial determinations of serum CA125 as well as pelvic examination increase the test specificity. Serum CA125 concentration may be useful in monitoring treatment and distinguishing between good response to treatment and progressive malignant disease with poor therapeutic response. To date, CA125 is the most sensitive marker for residual epithelial ovarian cancer. CA125 may also be elevated in patients with lung, cervical, fallopian tube, and uterine cancer and endometriosis.

PRINCIPLE OF THE TEST
The ERBAlisa CA 125 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
Microwells coated with Streptavidin	12x8x1
CA 125 Standard Set: 6 Vials (ready to use)	0.5 ml x 6
CA 125 Enzyme Conjugate: 1 Bottle (ready to use)	12 ml
TMB Substrate: 1 Bottle (ready to use)	12 ml
Stop solution: 1 Bottle (ready to use)	12ml
20X Wash Concentrate: 1 Bottle	25 ml

MATERIALS NOT PROVIDED
1. Distilled or deionized water
2. precision pipettes
3. Disposable pipette tips
4. Micortiter well 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 test reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS
1. Potential biohazardous materials: The calibrator and controls 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. This test kit is designed for IVD Use.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. 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 HANDLING
1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8° C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION

Wash buffer: 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.

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 OF RESULTS

The standard curve is constructed as follows:

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

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Example of a 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

EXPECTED VALUES

We recommend each laboratory to establish its own normal ranges, for the population it serves. Until then, literature values may be used as guidelines.

Healthy and Non-Pregnant Individual	≤46 (U/mL)

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

PERFORMANCE CHARACTERISTICS

1. **Sensitivity:** The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 22 times in the same run.

Serum	No. of Replicates	Mean (U/ml)	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	22	0.10	0.208	0.52 U/ml

2. **Correlation with a Reference ELISA kit:**
   Serum samples were tested by this ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.98	1.0447	0.9216

3. **Precision**

Intra-Assay

Serum	No. of Replicates	Mean - U/ml	Standard Deviation	Coefficient of Variation (%)
1	16	22.6	0.937	4.2
2	16	67.9	1.29	1.9
3	16	152	2.83	1.9

Inter-Assay

Serum	No. of Replicates	Mean - U/ml	Standard Deviation	Coefficient of Variation (%)
1	24	19.6	1.61	8.2
2	24	56.9	3.88	6.8
3	24	135.6	9.06	6.7

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