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.

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CA125 ELISA

Catalog No. IB19118 (96 Tests)

INTENDED USE

The CA125 ELISA Kit is intended for the quantitative determination of CA125 concentration in human serum. For research use only. Not for use in diagnostic procedures.

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 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 Reagent (One-Step)	12 ml
6. Stop Solution	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

1. For research use only. Not for use in diagnostic procedures.
2. For laboratory use only.
3. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different kit lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care since it is corrosive.
15. Bring all reagents to room temperature.
16. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
17. Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

SAMPLE COLLECTION AND PREPARATION

Serum should be prepared from a whole blood sample obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

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, samples, 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 at room temperature (20-25 °C).
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 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 (450nm)
0	0.010
15	0.105
50	0.347
100	0.703
200	1.411
400	2.437