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CA 15-3 ELISA Kit

Name	CA 15-3 ELISA Kit
INTENDED USE	The CA15-3 ELISA Kit is intended for the quantitative determination of the cancer antigen CA15-3 concentration in human serum or plasma.
SUMMARY AND EXPLANATION	Breast cancer is the most common life-threatening malignant lesion in women of developed countries today, with approximately 180,000 new cases diagnosed every year. Roughly half of these newly diagnosed patients are node-negative, however 30% of these cases progress to metastatic disease. There are a number of tumor markers that can help clinicians to identify and diagnose which breast cancer patients will have a more aggressive disease. These markers include estrogen and progesterone receptors, DNA ploidy and percent-S phase profile, epidermal growth factor receptor, HER-2/neu oncogene, p53 tumor suppressor gene, cathepsin D, proliferation markers and CA15-3. CA15-3 is most useful for monitoring patients post-operatively for recurrence, particularly in metastatic disease cases. 96% of patients with local and systemic recurrence have elevated CA15-3, which can be used to predict recurrence earlier than radiological and clinical criteria. A 25% increase in the serum CA15-3 is associated with progression of carcinoma. A 50% decrease in serum CA15-3 is associated with response to treatment. CA15-3 is more sensitive than CEA in early detection of breast cancer recurrence. In combination with CA125, CA15-3 has been shown to be useful in early detection of relapse of ovarian cancer. CA15-3 levels are also increased in colon, lung and hepatic tumors.
PRINCIPLE OF THE TEST	The CA15-3 ELISA test is an adapted solid phase sequential sandwich ELISA. Samples and biotinylated

monoclonal antibody are added to wells coated with streptavidin. CA15-3 in the patient sample binds to biotinylated capture antibody. The biotinylated antibody simultaneously binds to the streptavidin coated plate. After a wash step, anti-CA15-3–HRP enzyme conjugate is added and forms a sandwich around captured CA15-3. Unbound antibodies are washed off. TMB substrate is added resulting in the development of a blue color. The concentration of CA15-3 is directly proportional to the color intensity developed. A standard curve is generated relating color intensity to CA15-3 concentration.

MATERIALS PROVIDED	96 Tests
Microwells coated with streptavidin	12x8x1
Anti CA15-3-Biotin Conjugate, 1 bottle (Ready to use)	12 ml
Anti CA15-3-HRP Enzyme Conjugate, 1 bottle (Ready to use)	12 ml
Sample Diluent, 1 bottle (Ready to use)	25 ml
CA 15-3 Standards, 6 vials (Ready to use)	0.5 ml
TMB Solution, 1 bottle (Ready to use)	12 ml
Stop Solution, 1 bottle (Ready to use)	12 ml
Wash Concentrate 20x, 1 Bottle	25 ml

MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

Graph paper

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	Microcentrifuge tubes
STORAGE AND STABILITY	1. Store the kit at 2-8 C. 2. Keep microwells sealed in a dry bag with desicants. 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	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 Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. It is recommended that standards, controls, and samples be run in duplicate. Optimal results will be obtained by exercising 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 PERPARATION	Serum or plasma should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum, plasma-EDTA, or plasma-heparin samples.
REAGENT AND SAMPLE PREPARATION	Immediately before testing, prepare samples by diluting using a 1:9 ratio in Sample Diluent provided. Example: add 50ul of sample to 450ul of Sample Diluent and mix well. Discard unused diluted samples. Do not dilute the

	standards. 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 (20-25C).
ASSAY PROCEDURE	Bring all specimens and kit reagents to room temperature (20-25C) and gently mix. Patient samples should be diluted 10-fold before use. (See Reagent and Sample Preparation above). do not dilute the standards. Secure the desired number of coated wells in the holder. Dispense 25 l of CA15-3 standards, diluted samples, and diluted controls into the appropriate wells. Add 100ul of Antibody-Biotin Conjugate Reagent (blue solution) to all wells. Gently mix for 20-30 seconds at 500-600 rpm. Incubate for 60 minutes at room temperature. Remove liquid from all wells. Wash each well three times with 350 L of 1X wash buffer. After each wash, sharply and firmly tap the upside down plate on absorbance paper or paper towels to remove residual droplets. Dispense 100l of Enzyme Conjugate (red solution) into each well. Incubate for 60 minutes at room temperature. Remove the contents and wash the plate 3x as described in step 5 above. Dispense 100 l of TMB Solution into each well. Incubate at room temperature for 15 minutes. Stop the reaction by adding 50l of Stop Solution to each well. Read the absorbance at 450nm (using a reference wavelength of 630nm) with a microtiter plate reader within 15 minutes.
CALCULATIONS AND RESULTS	Calculate the average absorbance values for each set of reference standards, control, and samples. 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.

Using the mean absorbance value for each sample, determine the corresponding concentration of CA15-3 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 CA15-3 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 for every test run.

CA15-3 Values (U/ml)	Absorbance (450 nm)
0	0.028
10	0.167
30	0.428
60	0.835
120	1.385
240	2.208

LIMITATIONS OF THE PROCEDURE

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.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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