**REFRENCES**

1. Aziz DC, Peter JB. DNA ploidy and cell-cycle analysis. Tools for assessment of cancer prognosis. J Clin Pathol 1991;5:422-38.
2. Clark GM, Dressler LG, Owens MA, Dounds G, Oldaker T, McGuire WL. Prediction of relapse or survival in patients with node-negative breast cancer by DNA flow cytometry. N Engl J Med 1989;320:627-33.
3. Elledge RM, McGuire WL. Prognostic factors and therapeutic decisions in axillary node-negative breast cancer. Annu Rev Med 1993;44:201-10.
4. Foekens JA, Rio C, Seguin P, et al. Prediction of relapse and survival in breast cancer patients by pS2 protein. Cancer Res 1990; 50-3832-7.
5. Isola J, Visakorp T, Holli K, Kallioniemi D. Association of p53 expression with other prognostic factors and long term survival in node-negative breast cancer. J Cell Biochem 1992;(Suppl 16D):101.
6. Kute TE, Shao ZM, Snugg NK, Long RT, Russell GB, Case LD. Cathepsin D as a prognostic indicator for node-negative breast cancer patients using both immunoassays and enzymatic assays. Cancer Res 1992;52-198-203.
8. McGuire WL, Tandon AK, Allred D, Chamnes GC, Clark GM. How to use prognostic factors in axillary node negative breast cancer patients. J Natl Cancer Inst 1990;82:1006-7.
9. Nicholson S, Richard J, Sainsbury C, et al. Epidermal growth factor receptor (EGFr): results of a 6 year follow up study in operable breast cancer with emphasis on the node-negative subgroup. Br J Cancer 1991;63:146-50.
10. Somerville JE, Clarke LA, Biggart JD. C-erb B-2 overexpression and histological type of in-situ and invasive breast carcinoma. J Clin Pathol 1992;45-16-20.

2017-04-13

Calbiotech Inc.,

CALBIOTECH
A life science company

# CA15-3 ELISA

Catalog No. CA240T (96 tests)

## INTENDED USE

The Calbiotech 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 |
|---|---|---|
| 1. | Microwells coated with streptavidin | 12x8x1 |
| 2. | Anti CA15-3-Biotin Conjugate, 1 bottle (Ready to use) | 12 ml |
| 3. | Anti CA15-3-HRP Enzyme Conjugate, 1 bottle (Ready to use) | 12 ml |
| 4. | Sample Diluent, 1 bottle (Ready to use) | 25 ml |
| 5. | CA 15-3 Standards, 6 vials (Ready to use) | 0.5 ml |
| 6. | TMB Solution, 1 bottle (Ready to use) | 12 ml |
| 7. | Stop Solution, 1 bottle (Ready to use) | 12 ml |
| 8. | 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
7. 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**

1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory use.
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.

**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**

1. 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**.
2. 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. Patient samples should be diluted 10-fold before use. (See Reagent and Sample Preparation above). **DO NOT DILUTE THE STANDARDS.**
2. Secure the desired number of coated wells in the holder. Dispense 25 µl of CA15-3 standards, diluted samples, and diluted controls into the appropriate wells.
3. Add 100ul of Antibody-Biotin Conjugate Reagent (blue solution) to all wells. Gently mix for 20-30 seconds at 500-600 rpm.
4. Incubate for 60 minutes at room temperature.
5. Remove liquid from all wells. Wash each well three times with 350 µL 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.
6. Dispense 100µl of Enzyme Conjugate (red solution) into each well.
7. Incubate for 60 minutes at room temperature.
8. Remove the contents and wash the plate 3x as described in step 5 above.
9. Dispense 100 µl of TMB Solution into each well.
10. Incubate at room temperature for 15 minutes.
11. Stop the reaction by adding 50µl of Stop Solution to each well.
12. Read the absorbance at 450nm (using a reference wavelength of 630nm) with a microtiter plate reader within 15 minutes.

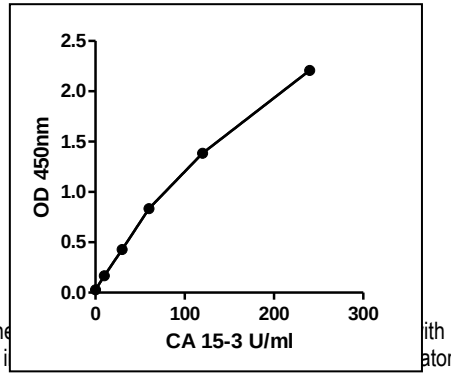
**CALCULATIONS AND RESULTS**

1. Calculate the average absorbance values 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 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**

1. Reliable and reproducible results will be obtaine ith a complete understanding of the package insert i atory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.