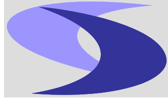


Signosis
BioSignal Capture

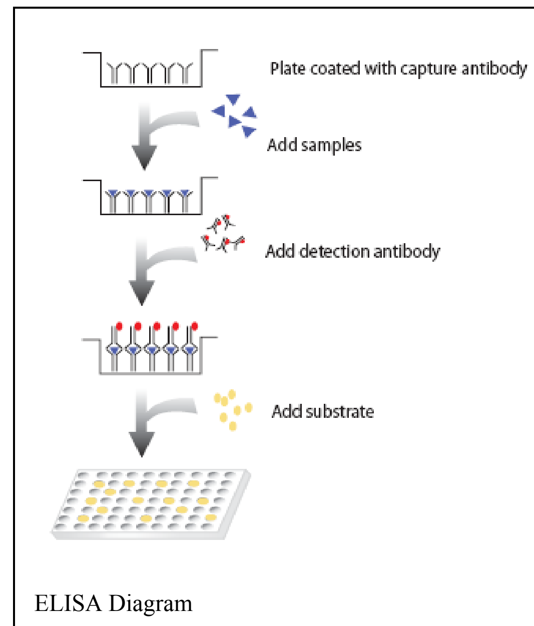
CA 15-3 ELISA

Catalog Number EA-0103 **(For Research Use Only)**

Introduction

CA 15-3 is expressed normally by most glandular epithelia but shows particularly increased expression in the breast at lactation and in malignancy(1). CA 15-3 is most useful in following the course of treatment in women diagnosed with breast cancer, especially advanced breast cancer and monitoring post operative patient for recurrence. CA 15–3 levels are rarely elevated in women with early stage breast cancer. Cancers of the ovary, lung, and prostate may also raise CA 15–3 levels. Elevated levels of CA 15–3 may be associated with noncancerous conditions, such as benign breast or ovarian disease, endometriosis, pelvic inflammatory disease, and hepatitis. Pregnancy and lactation can also cause CA 15–3 levels to rise (2-6).

The Signosis CA 15-3 Immunoassay provides a rapid, sensitive, and reliable assay for the quantitative measurement of CA 15-3 level. The antibodies developed for the test will determine a minimal concentration of CA15-3 in this assay is estimated to be 5 U/ml.



ELISA Diagram

Principle of the assay

The CA 15-3 ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal anti-CA 15-3 antibody directed against intact CA 15-3 for solid phase immobilization (on the microtiter wells). A rabbit anti-CA15-3 antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with these antibodies, resulting in CA 15-3 being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of CA 15-3 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Materials provided with the kits:

- Monoclonal Anti-CA15-3 coated microtiter plate with 96 well
- Sample diluent, 100ml
- Reference standard set, contains 0, 5, 20, 50, 150, and 300 ng/ml (WHO, 72/225) AFP, lyophilized
- Enzyme Conjugate (22X), 1.0 ml
- Enzyme Conjugate Diluent, 21 ml
- Wash Buffer (20X), 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Specimen Collection and Handling

Serum should be separated from the red blood cells as soon as possible. Specimens should be stored for up to 48 hours or -20°C for up to 6 months prior to assay.

Preparation for Assay

- All reagents should be taken to room temperature (18-25 °C) before use.
- Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8 °C.

Assay Procedure

Samples of patient serum, plasma need to be diluted before use for best bet results

1. Add 20 µl of standard, specimens, and controls into appropriate wells.
2. Add 100 µl of Zero Buffer into each well.
3. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this setup.
4. Incubate at room temperature (18-25ºC) for 30 minutes.
5. Remove the incubation mixture by flicking plate content into a waste container.
6. Rinse and flick the microtiter wells 5 times with Wash Buffer (1X). Put the wells sharply onto paper towel to remove all residual water droplets.
7. Add 150 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
8. Incubated at room temperature for 30 minutes.
9. Remove the incubation mixture by flicking plate contents into a waster container.
10. Rinse and flick the microtiter wells 5 times with Wash Buffer (1X).
11. Tap the plate sharply onto paper towel to remove residual water droplets.
12. Dispense 100 µl TMB Reagent into each well, and mix gently for 10 seconds.
13. Incubate at room temperature in the dark for 20 minutes.
14. Add 100 µl Stop Solution to each well to stop the reaction.
15. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
16. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

References

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Example of standard curve

CA15-3 Values (U/ml)	Absorbance (450 nm)
0	0.021
15	0.425
30	0.693
60	1.214
120	1.956
240	2.845