

# CA15-3 ENZYME IMMUNOASSAY TEST KIT

Catalog Number: EA100979



## Enzyme Immunoassay for the Quantitative Determination of CA15-3 in Serum

**FOR RESEARCH USE ONLY**  
**NOT FOR USE IN DIAGNOSTIC PROCEDURES**

### PRINCIPLE OF THE TEST

The CA15-3 ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA15-3 molecule is used 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 sequentially with the two antibodies, resulting in the CA15-3 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After two separate 1-hour incubation steps at 37°C, the wells are washed with Wash Buffer to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of CA15-3 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

### REAGENTS

#### Materials provided with the kit:

- Murine Monoclonal Anti-CA15-3 coated microtiter plate with 96 wells.
- Sample Diluent, 100 ml
- Enzyme Conjugate Concentrate (22x), 1.0 ml
- Enzyme Conjugate Diluent, 21 ml
- CA15-3 reference standards, containing 0, 15, 30, 60, 120, and 240 Unit/ml. Liquid, 1 set. **These standards have been pre-diluted 51-fold. Please do not dilute them again.**
- Wash Buffer Concentrate (20X), 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

### Storage of Test Kit and INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

### REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. To prepare working CA 15-3 Conjugate Reagent: Add the entire 1.0 ml of Conjugate Concentrate (22x) to 21 ml of the Enzyme Conjugate Diluent (1:21 dilution) and mix well. The diluted Enzyme Conjugate Reagent is stable at 4° C for at least 4 months.
3. To prepare **Wash Buffer (1X)**: Add 50 ml of Wash Buffer (20X) to 950 ml of DI water. The diluted Wash Buffer is stable at 2-8°C for 30 days. Mix well before use. Note: Any crystals that may be present due to high salt concentration must be redissolved at room temperature before making the dilution.

### ASSAY PROCEDURE

1. **Sample and control serum should be diluted, 51 fold, before use. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 20 µl serum with 1.0 ml Sample Diluent. PLEASE DO NOT DILUTE THE STANDARDS.**
2. Secure the desired number of coated wells in the holder.
3. Dispense 200 µl of CA15-3 standards, **diluted** specimens, and **diluted** controls into the appropriate wells. Gently mix for 10 seconds.
4. Incubate at 37°C for 1 hour.
5. Remove the incubation mixture by emptying the plate content into a waste container.
6. Rinse and empty the microtiter plate 5 times with **Wash Buffer (1X)**.
7. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 200 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds
9. Incubate at 37°C for 1 hour.
10. Remove the contents and wash the plate as described in steps 6-7 above.
11. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
12. Incubate at room temperature in the dark for 20 minutes.
13. Stop the reaction by adding 100 µl of Stop Solution to each well.
14. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
15. Read the optical density at 450 nm with a microtiter plate reader **within 15 minutes.**

## CALCULATION OF RESULTS

1. Calculate the average absorbance values ( $A_{450}$ ) 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.

## TECHNICAL CONSULTATION

**OriGene Technologies, Inc.**  
9620 Medical Center Dr., Suite 200  
Rockville, MD 20850

Phone: 1.888.267.4436

Fax: 301-340-9254

Email: [techsupport@origene.com](mailto:techsupport@origene.com)

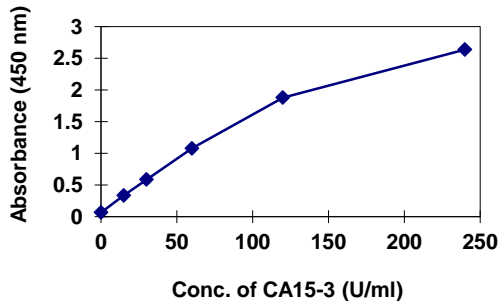
Web: [www.origene.com](http://www.origene.com)

## EXAMPLE OF STANDARD CURVE

091815

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against CA15-3 concentrations (U/ml) 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.

CA15-3 Values (U/ml)	Absorbance (450 nm)
0	0.067
15	0.338
30	0.587
60	1.081
120	1.880
240	2.640



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