AccuDiag™ CA-15-3 ELISA Kit

REF 6333-16

IVD See external Label 2-8°C Σ 96 Tests

Enzyme Immunoassay for the Quantitative Measurement of Breast Cancer Antigen (CA15-3) in Human Serum.

Test	CA-15-3 ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	0-240U/mL
Sample	100µL Serum
Specificity	97.5 %
Sensitivity	5U/mL
Total Time	~ 140 min
Shelf Life	12 Months from the manufacturing date

INTENDED USE

The CA15-3 ELISA test is intended for use as a monitoring and screening test for breast cancer. An abnormal result (i.e., elevated serum CA15-3 level) indicates further clinical management. CA15-3 is a useful tumor marker for patients in clinical remission following treatment. Post-operative serum CA15-3 values which fail to return to normal, strongly suggest the presence of residual tumor, while tumor recurrence is often accompanied by a rise of serum CA15-3 before progressive disease is clinically evident.

SUMMARY AND EXPLANATION

Breast cancer is the most common life-threatening malignant lesion in women of many 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.
T
here are a number of tumor markers that can help clinicians to identify and diagnose which breast cancer patients will have an aggressive disease and which will have an indolent course. 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 metastatic diseases. 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 are more sensitive than CEA in early detection of breast cancer recurrence. In combination with CA-125, 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.

TEST PRINCIPLE

The CA15-3 ELISA test is a two-site solid phase enzyme immunoassay. The molecules of CA15-3 are "sandwiched" between two monoclonal antibodies. One coated to the bottom of the wells of the microtiter plates and the other linked to the horseradish peroxidase (enzyme conjugate). After incubation and washing, the enzymatic reaction develops a color which is proportional to the amount of CA15-3 molecules present in the assay.

SPECIMEN COLLECTION AND PREPARATION

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. Specimens should be capped and may be stored for 48 hour at 2-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C for 6 months prior to assaying. Thawed samples should be inverted several times to mix prior to testing.

MATERIALS AND COMPONENTS

Materials provided with the test kits

- Monoclonal Anti-CA15-3 antibody coated microtiter plate with 96 wells.
- Sample diluent, 100 ml.
- Enzyme conjugate reagent, 12 ml.
- CA15-3 reference standard set, containing 0, 15, 30, 60, 120, and 240 Unit/ml (liquid, ready for use) or lyophilized form.
- Wash Buffer Concentrate (50X), 15 ml.
- TMB Substrate, 12 ml.
- Stop solution, 12 ml.
- Control set (optional)

Materials required but not provided

- Precision pipettes and tips, 0.1 ml, 0.2 ml, 1 ml, and 5 ml.
- Distilled water.
- Disposable pipette tips.
- Vortex mixer.
- Absorbent paper or paper towel.
- A microtiter plate reader at 450nm wavelength, with a bandwidth of 10nm or less and an optical density range of 0-2.5 OD or greater.
- Graph paper.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-22°C) before use. All reagents should be mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. If reference standards are lyophilized, reconstitute each standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.
3. Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, dilute 15 ml of Wash Buffer Concentrate

(50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

Important Note:

- ***The CA15-3 standards have already been prediluted and are ready for use.***

Please DO NOT dilute again!

1. Patient serum 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.
2. Secure the desired number of coated wells in the holder. Dispense 100µl of CA15-3 standards, diluted specimens, and diluted controls into the appropriate wells. Gently mix for 10 seconds.
3. Incubate at 37°C for 1 hour.
4. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with 1x wash buffer. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
5. Dispense 100 µl of enzyme conjugate reagent into each well. Gently mix for 10 seconds
6. Incubate at 37°C for 1 hour.
7. Remove the contents and wash the plate as described in step 4 above.
8. Dispense 100 µl TMB substrate reagent into each well. Gently mix for 10 seconds.
9. Incubate at room temperature for 20 minutes.
10. Stop the reaction by adding 100 µl of Stop Solution to each well. Gently mix for 10 seconds ensuring that the blue color completely changes to yellow.
11. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

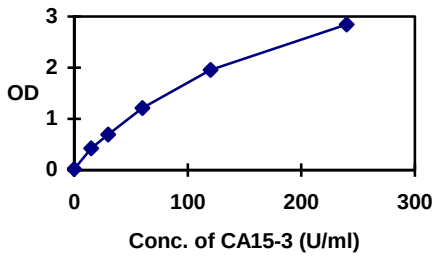
Important Note:

- ***The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.***
- ***It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.***
- ***Duplication of all standards and specimens, although not required, is recommended.***

RESULTS

Calculate the mean absorbance value for each set of CA15-3 reference standards, specimens and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in units per ml on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of CA15-3 in units per ml from the standard curve. It is recommended that samples be analyzed in duplicates. **Since the CA15-3 standards have already been diluted 51-fold, there is no need for the samples or controls to be multiplied by the dilution factor.**

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



LIMITATIONS OF THE PROCEDURE

There are some limitations of the assay:

1) As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2) Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.

STORAGE

1. 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. The test kit may be used throughout the expiration date of the kit (one year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2.5 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

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Diagnostic Automation/ Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax : 818-591-8383
Email: onestep@rapidtest.com Website: www.rapidtest.com

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