Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

CE

# AccuDiag™ CA-15-3 ELISA Kit

**Cat# 6333-16**

IVD See external Label 2°C–8°C 96 Tests

| 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 | 12-14 Months from the manufacturing date |

## INTENDED USE

The CA15-3 EIA 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.
There are a number of tumor markers that can help clinicians to identify and diagnose which breast cancer patients will have 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 EIA 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**

1. Monoclonal Anti-CA15-3 antibody coated microtiter plate with 96 wells.
2. Sample diluent, 100 ml.
3. Enzyme conjugate reagent, 12 ml.
4. CA15-3 reference standards, containing 0, 15, 30, 60, 120, and 240 Unit/ml. 1ml, liquid, ready for use. 1 set.
5. TMB Substrate, 12 ml.
6. Stop solution, 12 ml.
7. 50XWash Buffer Concentrate, 15 ml.

**Materials required but not provided**

1. Precision pipettes and tips, 0.1 ml, 0.2 ml, 1 ml, and 5 ml.
2. Distilled water.
3. Disposable pipette tips.
4. Vortex mixer.
5. Absorbent paper or paper towel.
6. A microtiter plate reader at 450nm wavelength, with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater.
7. 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. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750ml 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 waste container. Rinse and empty the microtiter plate 5 times with Washing buffer (1X) . 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. Dispense **100** µl TMB substrate reagent into each well. Gently mix for 10 seconds.
8. Incubate at room temperature in the dark for 20 minutes.
9. 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.
10. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

***Important Note*** :

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. 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.
3. Duplication of all standards and specimens, although not required, is recommended.

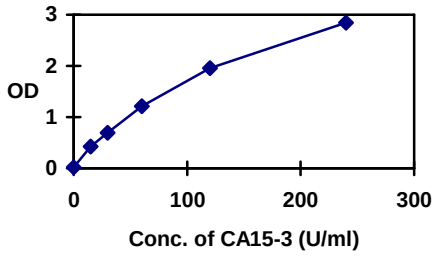
## RESULTS

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.**

**Example of Standard Curve**

Results of typical standard run with optical density reading at 450nm shown in the Y axis against CA15-3 concentrations shown in the X axis.

| 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 |




## PERFORMANCE CHARACTERISTICS

**I. Accuracy:** Comparison between Our Assay and Commercial Available Kits provides the following data

N = 55
Correlation Coefficient = 0.975
Slope = 0.924
Intercept = 1.92
Mean (Our Kits) = 28.93
Mean (Abbott) = 26.78

**II. Precision.**

**1]. Intra-Assay:**

| Concentrations | N | Mean | S.D. | % CV |
|---|---|---|---|---|
| Level I | 20 | 12.17 | 0.870 | 7.15 |
| Level II | 20 | 108.26 | 5.129 | 4.73 |

2]. Inter-Assay

| Concentrations | N | Mean | S.D. | % CV |
|---|---|---|---|---|
| Level I | 10 | 11.89 | 1.098 | 9.23 |
| Level II | 10 | 106.32 | 6.460 | 6.07 |

**III. Linearity**

Two patient sera were serially diluted with 0 U/mL standard in a linearity study. The average recovery was 102.5 %.

| Sample A | | | |
|---|---|---|---|
| **Dilution** | **Expected** | **Observed** | **% Recov.** |
| undiluted | 180.81 | 180.81 | |
| 2x | 90.41 | 88,92 | 98.4 |
| 4x | 42.21 | 43.72 | 103.6 |
| 8x | 22.60 | 23.03 | 101.9 |
| 16x | 11.30 | 12.04 | 106.5 |
| | | *Average Recovery: 102.6 %* | |

| Sample B | | | |
|---|---|---|---|
| **Dilution** | **Expected** | **Observed** | **% Recov.** |
| undiluted | 207.19 | 207.19 | |
| 2x | 103.60 | 105.23 | 101.6 |
| 4x | 51.80 | 50.33 | 97.2 |
| 8x | 25.90 | 26.67 | 103.0 |
| 16x | 12.95 | 13.91 | 107.4 |
| | | *Average Recovery: 102.3 %* | |

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**IV. Sensitivity**

The sensitivity is defined as the concentration of CA 15-3 that corresponds to the absorbance that is two standard deviations greater than the mean absorbance of 20 replicates of the zero calibrator. The minimum detectable concentration of this assay is estimated to be 5.0 U/mL.

**V. Cross-reactivity**

The following cancer marker antigens at high concentrations, as seen in cancer patients, were assayed to determine the possible reactivity.

| Antigens | Concentration | Equivalent CA 15-3 | % Cross-reactivity |
|---|---|---|---|
| CA-125 | 1,000 U/mL | 0.00 | 0.00 |
| CA 19-9 | 1,000 U/mL | 0.00 | 0.00 |
| PSA | 1,000 ng/mL | 0.00 | 0.00 |
| PAP | 1,000 ng/mL | 0.00 | 0.00 |
| AFP | 10,000 ng/mL | 0.00 | 0.00 |
| CEA | 1,000 ng/mL | 0.00 | 0.00 |

**VII. Hook Effect**

No hook effect at concentrations as high as 10,000 U/mL.

## LIMITATIONS OF THE PROCEDURE

1. 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.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Heterophilic antibodies such as human anti-mouse antibodies (HAMA) are frequently found in the serum of human subjects. Those antibodies can cause severe interference in many immunodiagnostic procedures. This assay has been designed to minimize that kinds of interference. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.

## 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 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

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| Date Adopted | Cat # 6333-16 |
|---|---|
| 2012-03-01 | AccuDiag™- CA-15-3 ELISA-2013 |
| EC REP | CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu |
| Revision Date: 10-25-2013 | |