IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards & samples is not mandatory but may provide information on reproducibility & application errors.

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. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

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SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	LOT Batch Number / Lot Number
Manufacturer	IVD *In vitro* Diagnostic Medical Device	This side up	Σ Contains sufficient for <n> tests
Use by	REF Catalogue Number	Do not reuse	

Manufactured by:

Zephyr Biomedicals

A Division of Tulip Diagnostics (P) Ltd.

M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

0422/VER-01

Qualisa™

Microwell Enzyme Immunoassay | CA 15-3

Breast Cancer Antigen

Enzyme Linked Immunosorbent assay for the Quantitative Determination of Breast Cancer Antigen (CA 15-3) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Qualisa™ CA 15-3 Sandwich ELISA test kit is intended for the quantitative determination of Breast Cancer Antigen (CA15-3) in Human Serum. For In Vitro Diagnostic Use only.

INTRODUCTION

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 CA 15-3. CA 15-3 is most useful for monitoring patients post-operatively for recurrence, particularly metastatic diseases. 96% of patients with local and systemic recurrence have elevated CA 15-3, which can be used to predict recurrence earlier than radiological and clinical criteria. A 25% increase in the serum CA 15-3 is associated with progression of carcinoma. A 50% decrease in serum CA 15-3 is associated with response to treatment. CA 15-3 is more sensitive than CEA in early detection of breast cancer recurrence. In combination with CA-125, CA 15-3 has been shown to be useful in early detection of relapse of ovarian cancer. CA 15-3 levels are also increased in colon, lung and hepatic tumors.

PRINCIPLE OF THE ASSAY

Qualisa™ CA 15-3 Quantitative Test Kit is a sandwich-based enzyme-linked immunosorbent assay. The assay system utilizes one monoclonal anti-CA 15-3 antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-CA 15-3 antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CA 15-3 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation the wells are washed and bound enzyme is detected by adding substrate. The reaction is stopped after specified time with stop solution and absorbance is determined for each well using an ELISA reader. The concentration of CA 15-3 is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with monoclonal anti- CA 15-3 antibody.
- Sample diluent
- Enzyme Conjugate. Ready to use.
- TMB Substrate. Ready to use
- Stop Solution. Ready to use
- CA 15-3 Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).

Materials required but not provided

- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **Qualisa™ CA 15-3** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to white at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

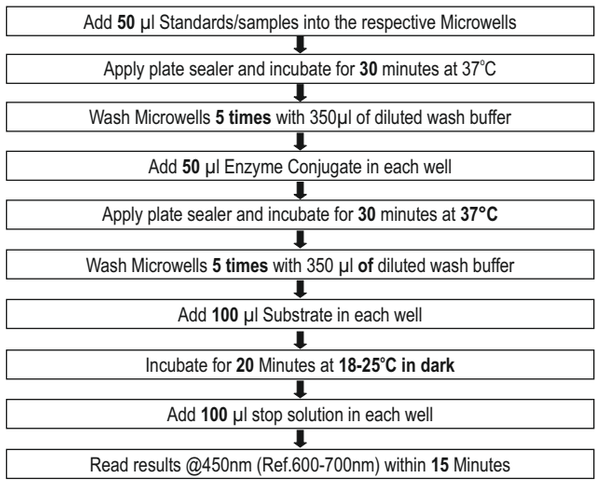
(1). Bring all reagents and specimen to room temperature before use. (2). Do not pipette any material by mouth. (3). Do not eat, drink or smoke in the area where testing is done. (4). Use protective clothing and wear gloves when handling samples. (5). Use absorbent sheet to cover the working area. (6). Immediately clean up any spills with sodium hypochlorite.(7).All specimens and standards should be considered potentially infectious and discarded appropriately.(8).Neutralize acid containing waste before adding hypochlorite. (9). Do not use kit after the expiry date. (10). Do not mix components of one kit with another. (11). Always use new tip for each specimen and reagent. (12). Do not allow liquid from one well to mix with other wells.(13). Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water), Mix well before use.

TEST PROCEDURE

1. Patient serum should be diluted 51 fold, before use. (i.e mix **10 µl** serum with **500 µl** Sample Diluent).
2. ***Important Note:*** **The CA15-3 standards have already been prediluted and are ready for use.** ***Please DO NOT dilute again.***
3. Secure the desired number of coated wells in the holder. Dispense **50 µl** of CA15-3 standards & diluted specimens into the appropriate wells. Gently mix for 10 seconds & Incubate at 37°C for **30 minutes**.
4. After incubation, empty the microtitre wells and wash the plate 5 times with 350µl of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
5. Dispense **50 µl** of Enzyme Conjugate into each well. Incubate at 37°C for **30 minutes**.
6. Remove the contents and wash the plate as described in step 4 above.
7. Dispense **100 µl** TMB Substrate into each well. Incubate at room temperature **(18-25°C)** in dark for **20 minutes**.
8. 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.
9. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes**.



CALCULATION OF RESULTS

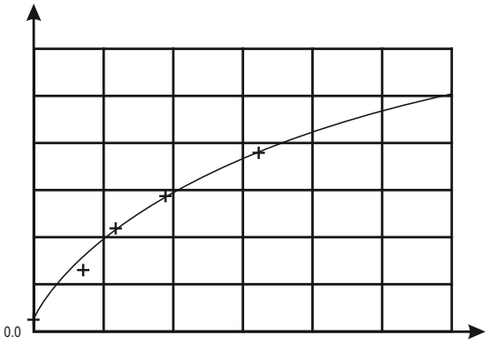
Construct a Standard curve by plotting the absorbance obtained from each reference Standard against its concentration in U/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of CA 15-3 in U/ml from the Standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve

Results of a typical standard run with optical density reading at 450nm (ref 600-700nm) shown in the Y axis against CA 15-3 concentrations shown in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

CA 15-3 Values (U/ml)	Absorbance
A	0.012
B	0.189
C	0.365
D	0.665
E	1.183
F	1.772



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 standard curve and data.

Expected values

Healthy women are expected to have CA 15-3 assay values below 35 U/ml.
The minimum detectable concentration of CA 15-3 in this assay is estimated to be 5 U/ml.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **Qualisa™ CA 15-3** was evaluated against commercially available licensed kit with 90 random clinical samples & **Qualisa™ CA 15-3** has demonstrated 100% clinical correlation with the commercially available licensed kit.
   Precision: **Qualisa™ CA 15-3** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with **Qualisa™ CA 15-3**	Coefficient of Variation (CV)
Level 1	10	21.11	6.60
Level 2	10	48.12	3.48
Level 3	10	96.14	1.36

B) External Evaluation:

Qualisa™ CA 15-3 ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Qualisa™ CA 15-3** ELISA has demonstrated 100% correlation with the reference method.
*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd.).