



## **Human MUC1 / EMA ELISA Kit**

Enzyme Immunoassay for the quantification of human MUC1 / EMA (CA 15-3) in serum and plasma.

Catalog number: ARG80644

Package: 96 wells

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For research use only. Not for use in diagnostic procedures.

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### INTRODUCTION

Breast cancer is one of the most common malignancies among women. Nine categories of breast tumor showed evidence of clinical utility and were recommended for use in practice. Among these, CA 15-3 which detects soluble forms of MUC-1 protein is the most widely used serum marker in patients with breast cancer. The main use of CA 15-3 is for monitoring therapy in patients with metastatic disease. Metastatic disease may be present at the time of initial diagnosis and can occur at any time following primary therapy. Up to 70% of patients with metastases will respond to systemic treatment with cytotoxic drugs or endocrine therapy; therefore, early detection of recurrence is important to patient management. In patients previously treated for stage II or stage III breast cancer, early detection of recurrence cannot be readily accomplished by routine clinical or diagnostic studies alone. The use of a circulating serum tumor marker assay, such as arigo CA 15-3 ELISA, can be useful in the identification of these patients. In addition, CA 15-3 may also be used in the postoperative surveillance of asymptomatic women who have undergone surgery for invasive breast cancer. Finally, preoperative concentrations of CA 15-3 might be combined with existing prognostic factors for predicting outcome in patients with newly diagnosed breast cancer.

CA 15.3, although preferentially associated with breast cancer, is not tissue specific, and was shown to be elevated in different varieties such as ovarian cancer and colon adenocarcinoma (9). CA 15-3 assay values were not elevated in the sera of the majority of normal individuals or those with nonmalignant

conditions.

**NOTE:** CA 15-3 values determined with different assays and from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Assay values obtained with different assay methods cannot be used interchangeably. In addition, the main limitation of CA 15-3 as a marker for breast cancer is that serum levels are rarely increased in patients with early or localized disease.

### **PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CA 15-3 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any CA 15-3 present is bound by the immobilized antibody. After washing away any unbound substances, a Horseradish Peroxidase (HRP)-conjugated antibody specific for CA 15-3 is added to each well and incubate. Following a washing to remove unbound substances, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of CA 15-3 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm  $\pm$  2 nm. The concentration of CA 15-3 in the sample is then determined by comparing the O.D of samples to the standard curve.

### MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C.
Zero standard	3 ml (ready to use)	4°C
Standard 1-4 (25, 50, 100, 200 U/ml)	4 x 0.5 ml (ready to use)	4°C
Control low (Acc. Range: 22.5-52.5 U/ml)	0.5 ml	4°C, lyophilized
Control high (Acc. Range: 54.7-128 U/ml)	0.5 ml	4°C, lyophilized
HRP conjugated antibody	14 ml (ready to use)	4°C
Assay Buffer	30 ml (ready to use)	4°C
40X Wash buffer	30 ml	4°C
TMB substrate	14 ml(ready to use)	4°C (Protect from light)
STOP solution	14 ml(ready to use)	4°C

### MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

### TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times. Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for 8 weeks if stored as described above.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 40X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Bring all reagents and required number of strips to room temperature prior to use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

## **SAMPLE COLLECTION & STORAGE INFORMATION**

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Serum**- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA, heparin or citrate plasma as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

Note:

- Samples containing sodium azide should not be used in the assay.
- Do not use haemolytic, icteric or lipaemic specimens.
- Thawed samples should be inverted several times prior to testing.

## **REAGENT PREPARATION**

- **1X Wash buffer:** Dilute **40X** Wash buffer into **distilled water** to yield 1X Wash buffer. Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL. The diluted Wash Solution is stable for 2 weeks at room temperature.
- **Controls:** Reconstitute the lyophilized content with **0.5 mL** of **distilled water** and let stand for 10 minutes in minimum. Mix the controls several times before use.

Note: The reconstituted controls are stable for 2 days at 2 °C to 8 °C. For longer storage the reconstituted controls should be apportioned and stored at -20 °C.

- **Samples:** If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with **Zero Standard** and reassayed as described in “Assay Procedure”. For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) Dilution 1:10: 10 µL sample + 90 µL Zero Standard (mix thoroughly)

b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Zero Standard (mix thoroughly).



### ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

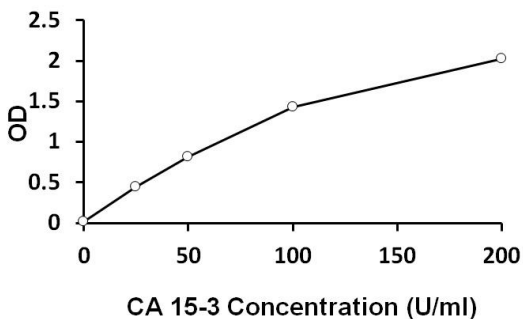
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **10 µl** of **standards, controls and samples** in duplicate into sample wells.
3. Add **250 µL** of **Assay Buffer** into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **60 minutes at RT.**
5. Aspirate each well and wash, repeating the process 3 times for a **total 4 washes**. Wash by filling each well with **1× Wash Buffer (400µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µl** of **HRP conjugated antibody** into each well. Incubate for **60 minutes at RT.**
7. **Wash** as according to **step 5.**
8. Add **100 µl** of **TMB substrate mixture** to each well. Incubate for **15 minutes at room temperature** in dark.
9. Add **100 µl** of **Stop Solution** to each well.
10. **Read** the OD with a microplate reader **at 450 nm** immediately. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

### CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using log-log, semi log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value 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 from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (<https://www.arigobio.com/elisa-analysis>)
6. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 200 U/mL. For the calculation of the concentrations this dilution factor has to be taken into account.
7. TEST VALIDITY: The following specifications must be met for each run to be valid:  
O.D. value for the highest value calibrator:  $\geq 1.200$   
If the specifications is not met, the test run should be repeated.

### EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



### QUALITY ASSURANCE

#### Sensitivity

The analytical sensitivity of CA 15-3 ELISA kit was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Zero Standard and was found to be 0.5 U/mL

#### Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7.3% and inter-assay precision was 10.77%.

#### Recovery

85-114.9%

#### Interfering Substances

Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 30 mg/ml) have no influence on the assay results.