

1. NAME AND INTENDED USE

ELSA-CA 15-3 is a kit for the immunoradiometric assay of the CA 15-3 antigen in human serum or plasma.

2. INTRODUCTION

CA 15-3 is a human breast tumor-associated antigen, recognized and assayed by two monoclonal antibodies. The first, 115D8*, was raised against antigens from human milkfat globule membranes and the second, DF3*, against a membrane-enriched fraction of a human breast carcinoma. The CA 15-3 antigen recognized by both of these monoclonal antibodies is a high molecular weight glycoprotein found at elevated levels in the sera of breast cancer patients.

High pre-operative levels of CA 15-3 are associated with a poor prognosis and correlate to the tumor volume. During a remission period, repeated testing for CA 15-3 enables an early diagnosis of a relapse or metastasis. When a diagnosis of metastasis has been made and throughout the treatment period, variations in CA 15-3 level are correlated to therapeutic efficiency.

The use of CA 15-3 assays is therefore indicated at all stages in the evolution of a breast carcinoma, though it must under no circumstances be considered a screening test.

The sensitivity at the time of diagnosis of metastatic breast cancer is close to 80%.

3. PRINCIPLE

ELSA-CA 15-3 is a solid phase "sandwich" immunoradiometric assay. Two monoclonal antibodies were prepared against two sterically-remote antigenic sites on the CA 15-3 molecule. The first is coated on the ELSA solid phase, and the second, radiolabeled with iodine 125, is used as a tracer.

CA 15-3 molecules present in the standards or the samples to be tested are "sandwiched" between the two antibodies. Excess tracer is easily removed during the washing step of the procedure, and only the adsorbed coated antibody/antigen/tracer antibody combination remains on the ELSA.

The amount of radioactivity bound to the ELSA is thus proportional to the amount of CA 15-3 present in the sample at the beginning of the assay.

4. REAGENTS

Each kit contains sufficient reagents for 96 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
ELSA : ready for use. Monoclonal anti-CA 15-3 antibody coated on the ELSA fixed at the bottom of the tube.	4 tray-packs of 24 tubes	2-8°C until the expiry date. ELSA tubes removed from their packs must be stored in the plastic bag supplied with the kit.
ANTI-CA 15-3 ¹²⁵I : ready for use. Anti-CA 15-3 ¹²⁵ I monoclonal antibody, buffer, calf serum, sodium azide, non-immunized mouse immunoglobulins, red dye. ≤ 555 kBq (≤ 15 µCi).	1 30 ml vial	2-8°C until the expiry date.
STANDARDS : lyophilized*, prediluted. Human CA 15-3 antigen, buffer, horse serum, bovine albumin, sodium azide. 0 - 15 - 40 - 80 - 140 - 240 U/ml. Reconstitute with 2.0 ml of distilled water.	6 2 ml vials	2-8°C until the expiry date. After reconstitution, store 1 month at 2-8°C or 2 months frozen at -20°C.
CONTROL : lyophilized** Human CA 15-3 antigen, buffer, horse serum, sodium azide. Expected value: 30 U/ml. Reconstitute with 0.2 ml of distilled water.	1 0.2 ml vial	2-8°C until the expiry date. After reconstitution, store 1 month at 2-8°C or 2 months frozen at -20°C.
DILUENT : ready for use. Buffer, bovine albumin, sodium azide.	1 100 ml vial	2-8°C until the expiry date.
PLASTIC BAG	1	

(*) The values shown above are only target values; the true value of each standard is shown on its label and is expressed in units/ml (arbitrary system based on a reference preparation).

(**) The acceptance range true values are printed on the vial label.



* Fujirebio Diagnostics Inc. Antibodies

5. PRECAUTIONS FOR USE

5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBS antigen. However, as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth.

Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.

Avoid splashing.

Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Basic radiation protection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

Enforcement of the basic radioprotection rules will ensure adequate security.

A summary of these is given below:

Radioactive products must be stored in their original containers in a suitable area.

A record of the reception and storage of radioactive products must be kept up to date.

Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).

Do not eat, drink, smoke or apply cosmetics in a controlled zone.

Do not mouth-pipette radioactive solutions.

Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.

Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.

Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.

All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions

Do not use kit components beyond their expiry date.

Do not mix reagents from different batches.

Avoid any microbic contamination of the reagents or of the water used for washing.

Fully respect the incubation times and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum or plasma. If the assay is to be carried out within 24 hours of sample collection, the samples may be stored at 2-8°C. Otherwise, they should be divided into aliquots and deep-frozen (-20°C).

Dilutions

When high CA 15-3 levels are suspected, dilution of the sample is performed with the diluent supplied in the kit.

The use of disposable plastic tubes is recommended for the dilution operations.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar, with disposable tips, permitting the dispensing of 20 µl, 300 µl and 1000 µl, 2000 µl (± 1%). Their calibration must be checked regularly.

Distilled water. Disposable plastic tubes. Vortex-type mixer. Orbital horizontal shaker. Gamma scintillation counter calibrated for 125 iodine measurement.

7.2. Protocol

All reagents must be brought to room temperature (18-25°C) in their unopened packaging at least 30 minutes before use. Dispensing of the reagents into the ELSA tubes is also to be carried out at room temperature. Open the cover of the control vial with caution and carefully reconstitute its contents according to the instructions (see n°4 REAGENTS).

The assay requires the following groups of tubes: 0 Standard group for the determination of non-specific binding, standard groups to establish the standard curve, control group for checks, Sx groups for the samples to be assayed. It is recommended that the assay be performed in triplicate for the standards and in duplicate for the samples.

Respect the order of addition for the reagents :

Predilution of samples and control (1/51). Dispense 20 µl of each sample or control into the plastic tubes. Add 1 000 µl of diluent to each tube and mix.

NOTE: after reconstitution, the standards are ready to use; do not predilute.

Dispense 300 µl of standards and control or prediluted samples into the corresponding ELSA tubes (check labels).

Gently mix each tube with a Vortex-type mixer.

Incubate for 1 hour ± 5 minutes at room temperature (18-25°C) while continuing shaking at 400 rpm.

Wash the ELSA tubes as follows:

Aspirate the contents of the tubes as completely as possible.

Add 3.0 ml of distilled water to each tube; re-empty.

Repeat the operation twice, for a total of 3 washes.

For reliable and reproducible results to be obtained, the different washing steps must be efficient: as much as possible of the different incubation and washing solutions must be removed. If they are carried out manually, the tip of the aspirating device must reach right to the bottom of the ELSA tube.

Add 300 µl of anti-CA 15-3 ¹²⁵I monoclonal antibody to all of the tubes. Mix gently (Vortex).

Incubate 1 hour ± 5 minutes at room temperature (18-25°C) while continuing shaking at 400 rpm.

Wash the ELSA tubes in the manner previously described.

Using a gamma scintillator, measure the radioactivity bound to the ELSA.

8. QUALITY CONTROL

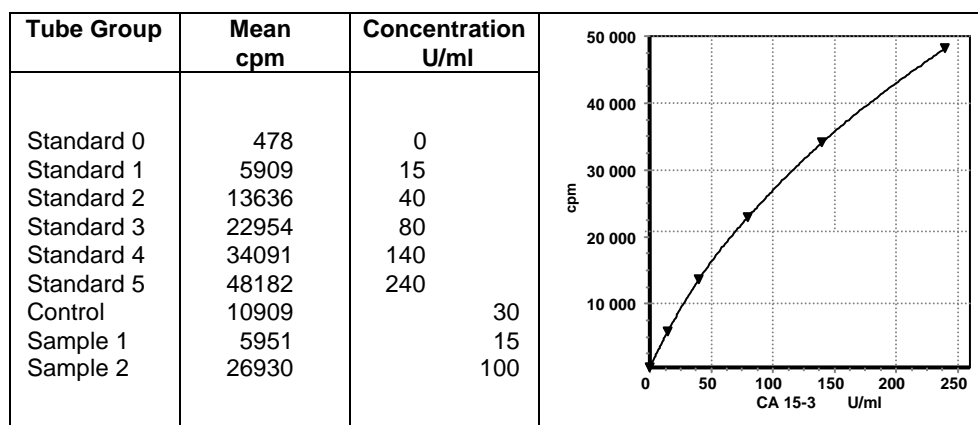
Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

For each group of tubes compute the mean counts after subtracting the background. Draw the standard curve, plotting the standards' cpm compared to their concentrations.

Read the sample values directly from the curve, correcting the read value for the dilution factor if necessary.

Typical standard curve (example only): This data must under no circumstances be substituted for results obtained in the laboratory.



10. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results.

Do not extrapolate values for samples beyond the last standard. Dilute the samples concerned and re-assay.

11. EXPECTED VALUES

Each laboratory must establish its own range of normal values. The values given below must only serve as an indicator.

A study concerning 186 women unaffected by any benign or malignant pathology has shown that 98.6% of the values were below 30 U/ml.

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Imprecision

This was evaluated using 2 samples with different concentrations assayed either 30 times in the same series or in duplicate in 10 different series.

Sample	Mean U/ml	Within-run CV %	Between-run CV %
1	41.4	5.2	4.8
2	105	5.4	6.0

12.2. Recovery test

Known quantities of CA 15-3 were added to human sera. Recovery percentages of CA 15-3 obtained ranged between 95 and 105%.

12.3. Dilution test

Ten high-value samples were diluted. Recovery percentages were between 95 and 115%.

12.4. Specificity

The antibodies used in this assay guarantee a measurement which is completely specific for CA 15-3.

12.5. Detection limit

The detection limit is defined as being the smallest concentration different from 0 with a confidence interval of 95%. It has been determined as being 0.2 U/ml.

12.6. Measurement range

0.2 – 240 U/ml.

ASSAY FLOW CHART

Tubes	Standards Control* Samples* µl	Mix. Incubate 1 hour at 18-25°C, continuing shaking.	125µl Anti-CA 15-3 µl	Mix. Incubate 1 hour at 18-25°C continuing shaking.	Measure
Standards	300	Wash 3 times.	300	Wash 3 times.	
Control or Samples	300		300		

(*) Control and samples must be prediluted.