

## 1. NAME AND INTENDED USE

ELSA-CA 72-4 is an immunoradiometric assay for the quantitative determination of TAG 72 antigen in human serum or plasma.

## 2. INTRODUCTION

TAG 72 is a high molecular weight glycoprotein associated with tumors, and recognized by B 72-3\* and CC 49 monoclonal antibodies\*.

The B 72-3 antibody was obtained by immunizing a mouse with a metastatic human breast cancer-enriched membrane fraction. In immunohistochemistry, it shows good affinity for gastro-intestinal and human mammary carcinomas, as opposed to the corresponding benign or normal tissues. The CC 49 antibody was generated after immunizing a mouse with TAG 72 previously purified by affinity chromatography. It recognizes a different epitope from that recognized by B 72-3.

This assay system was used in numerous clinical studies, which demonstrated its good level of sensitivity to gastric cancers (70% for the metastatic stages and 20% for the non-metastatic) and its outstanding specificity.

The assay also enables the follow-up of gastric cancer cases under treatment or in remission.

## 3. PRINCIPLE

ELSA-CA 72-4 is a solid phase two-site immunoradiometric assay. Two monoclonal antibodies were prepared against sterically remote antigenic sites on the TAG 72 molecule : the first is coated on the ELSA solid phase, the second, radiolabeled with iodine 125, is used as a tracer.

TAG 72 molecules present in the standards or the samples to be tested are "sandwiched" between the two antibodies. Following the formation of the coated antibody/antigen/iodinated antibody sandwich, the unbound tracer is easily removed by a washing step.

The radioactivity bound to the ELSA is proportional to the concentration of TAG 72 present in the sample.

## 4. REAGENTS

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

REAGENTS	QUANTITY	STORAGE
<b>ELSA:</b> ready for use. Monoclonal anti-CA 72-4 antibody coated on ELSA fixed to the bottom of the tube.	4 traypacks of 24 tubes	2-8°C until the expiry date. Tubes removed from their packs must be stored in the bag supplied with the kit.
<b>ANTI-CA 72-4 <sup>125</sup>I:</b> ready for use. Monoclonal anti CA 72-4 <sup>125</sup> I antibody, buffer, bovine albumin, preservative, red dye, non immunized mice immunoglobulins. ≤ 370 kBq (≤ 10 µCi).	1 30 mL vial	2-8°C until the expiry date. After opening, 15 days at 2-8°C.
<b>STANDARDS:</b> ready for use. Human serum, human TAG 72, sodium azide, 3 - 10 - 25 - 50 - 100 U/mL*.	5 0.8 mL vials	2-8°C until the expiry date. After opening, 15 days at 2-8°C.
<b>CONTROL:</b> ready for use. Human serum, human TAG 72**, sodium azide.	1 0.8 mL vial	2-8°C until the expiry date. After opening, 15 days at 2-8°C.
<b>DILUENT:</b> ready for use. Buffer, bovine albumin, human TAG 72, sodium azide, 3 U/mL*.	1 10 mL vial	2-8°C until the expiry date.
<b>BUFFER:</b> ready for use. Buffer, bovine albumin, sodium azide.	1 20 mL vial	2-8°C until the expiry date.
<b>PLASTIC BAG</b>	1	

(\*) CA 72-4 concentration is expressed in units/ml (arbitrary system based on a reference preparation).  
The values shown above are only target values ; the true value of each standard is shown on its label.

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

## 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 radioprotection 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.

The enforcement of the basic rules for handling radioactive products ensures 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 conditions and the washing instructions indicated.

## 6. SPECIMEN COLLECTION AND PREPARATION

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

### Dilutions

Should elevated CA 72-4 levels be suspected, dilution is performed with the diluent found in the kit.

It is recommended that disposable plastic tubes be used when carrying out dilutions.

## 7. ASSAY PROCEDURE

### 7.1. Material required

Precision micropipettes or similar, with disposable tips, capable of dispensing 100 µL, 200 µL and 300 µL (± 1%). Their calibration should be checked regularly.

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

### 7.2. Protocol

All reagents except the <sup>125</sup>I anti CA 72-4 monoclonal antibody must be brought to room temperature (18-25°C) at least 30 minutes before their use. Dispensing of the reagent into the ELSA tubes is also carried out at room temperature.

The assay requires the following groups of tubes:

Standard groups to establish the standard curve.

Control group for the control.

Sx groups for the test samples.

It is recommended that the assay be performed in triplicate for the standards and in duplicate for the samples.

Respect the order in which reagents are to be added:

Dispense 200 µL of buffer into all the ELSA tubes.

Add 100 µL of standards, control or samples to the corresponding groups of tubes.

Gently mix each tube with a Vortex-type mixer.

Incubate 4 hours ± 5 mn at room temperature (18-25°C) under continuous shaking (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, and re-empty.

Repeat this process twice.

To obtain reliable and reproducible results, the different washing steps have to be correctly performed. As much as possible of the incubation and washing solutions must be removed. If the washing is carried out manually, the tip of the aspirating device must be placed right at the bottom of the ELSA tube.

Add 300 µL of anti-CA 72-4 <sup>125</sup>I monoclonal antibody to all the tubes.

Gently mix each tube with a Vortex-type mixer.

Incubate overnight (16-20 h) at 2-6°C.

The incubation temperature should not exceed 6°C.

Wash the ELSA tubes as previously described.

Measure the remaining radioactivity bound to the ELSA with a gamma scintillation counter.

## 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

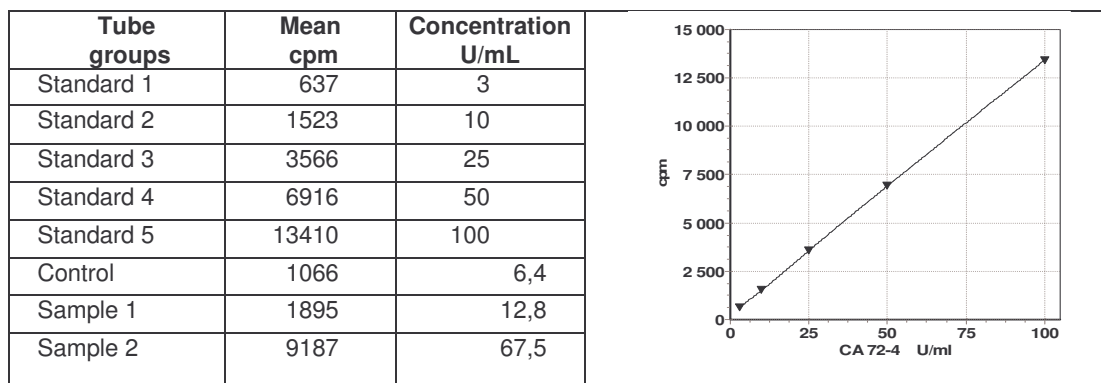
For each group of tubes, calculate the mean counts after subtracting the background.

Draw up the standard curve by plotting the standard's cpm against their concentrations.

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

Do not forget to subtract the diluent CA 72-4 concentration to obtain the final concentration of the specimens.

**Typical standard curve** (example only) : this data must not 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 sample values beyond the last standard. Dilute the samples concerned and re-assay.

## 11. EXPECTED VALUES

Each laboratory should establish its own range of normal values.

A study measuring the level of CA 72-4 in 200 presumably healthy, smoker or non smoker subjects from both sexes has shown that 98.5% of the values were below 4 U/mL.

## 12. SPECIFIC CHARACTERISTICS OF THE ASSAY

### 12.1. Imprecision

This has been assessed using 2 samples with different concentrations. They were tested either 30 times in the same series of assays, or in duplicate in 10 different series.

Sample	Mean U/mL	Within-run CV %	Between-run CV %
1	9	9.3	11.7
2	83	5.1	5.7

### 12.2. Recovery test

Known quantities of CA 72-4 were added to human sera. The recovery percentages of CA 72-4 in the samples ranged from 90 to 110%.

### 12.3. Dilution test

Ten samples with high levels were diluted, with the recovery percentages ranging from 90 to 110%.

### 12.4. Specificity

The antibodies used in this assay guarantee a measurement which is completely specific for TAG 72.

### 12.5. Detection limit

It has been assessed as being 0.8 U/mL.

## ASSAY FLOW CHART

Tubes	Buffer $\mu$ L	Standards Control or Samples $\mu$ L	Mix gently. Incubate 4 hours at 18-25°C under agitation (400 rpm) Wash 3 times.	$^{125}$ I anti CA 72-4 $\mu$ L	Mix gently. Incubate overnight (16-20 h) at 2-6°C. Wash 3 times.	Count
Standards	200	100		300		
Control	200	100		300		
Samples	200	100		300		