RK-724CTACE160401

CA72-4 [I-125] IRMA KIT

(REF: RK-724CT and RK-724CT50)

The CA72-4 IRMA system provides a direct *in vitro* quantitative determination of the TAG-72 antigen (tumor associated glycoprotein 72) in human serum in the range of 3-100 U/mL. Each kit contains material sufficient for 100 or 50 assay tubes, permitting the construction of one standard curve and the assay of 44 (RK-724CT) or 19 (RK-724CT50) unknowns in duplicates.

Introduction

TAG-72 (also referred as CA72-4) is a high molecular weight, mucin-like glycoprotein which is predominantly expressed in malignant tumors of the gastrointestinal tract. TAG-72 was originally recognized by the murine monoclonal antibody B72.3, obtained after immunization with an enriched fraction of metastatic mammary carcinoma (Colcher et al, 1981). The purified antigen was used for raising second generation monoclonal antibodies, of which CC49 was selected for developing a double-determinant immunoassay (Gero et al, 1989). Both antibodies recognize different oligosaccharide epitopes on the TAG-72 molecule.

This assay system has been used in several clinical studies, which demonstrated its good sensitivity and specificity to gastric cancer. Elevated serum levels of TAG-72 have also been reported in carcinomas of the ovary, pancreas, colon, gallbladder, breast, cervix and endometrium. CA 72-4 is the marker of choice for the therapeutic monitoring and follow-up of gastric cancer patients.

Principle of method

The technology uses two monoclonal antibodies of high affinity in an immunoradiometric assay (IRMA) system. This assay is based on a two-step procedure. In the first step the serum sample is incubated in streptavidin coated tubes with biotin labeled, capture monoclonal antibody (CC49*). During this incubation period with continuous agitation the immuno-complex is immobilized on the reactive surface of the test tubes. After incubation the tubes are washed.

In the second stage ^{125}I-labelled, signal monoclonal antibody (B72.3*) is added and it binds to an epitope of the CA72-4 molecule different from that recognised by the capture antibody, resulting in the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

The reaction mixture is then discarded, the test tubes washed exhaustively, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in the test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of CA72-4, the unknown concentration of CA72-4 in patient samples can be determined.

*Fujirebio Diagnostics Inc. antibodies

FUJIREBIO Diagnostics, Inc.

Contents of the kit

1. One bottle of TRACER (21 mL), ready to use, containing < 980 kBq ^{125}I-B72.3 antibody in buffer with red dye and 0.1 % NaN_3.
2. One bottle of ANTISERUM (11 mL), ready to use, containing biotinilated CC49 antibody in buffer with blue dye and 0.1 % NaN_3.
3. One bottle (5 mL) of DILUENT containing equine serum and PBS buffer with 0.1% NaN_3.
4. Four vials of STANDARDS (4 x 1 mL), S1-S4, containing app. 3-20-50-100 U/mL CA72-4 in human serum with 0.1% NaN_3. The concentrations of standards are specified in the quality certificate enclosed. *Assay calibration was performed using Fujirebio Diagnostics Inc. CA72-4RIA.*
5. Two vials of CONTROL SERA (2 x 1 mL), CI-CII, containing low-positive and high-positive CA72-4 concentrations in human serum with 0.1% NaN_3. The concentrations of controls are specified in the quality certificate enclosed.
6. COATED TUBES, ready to use.

   Reactive test tubes, 12x75 mm, packed in plastic boxes. (**RK-724CT**: 2 boxes, 2x50 pcs; **RK-724CT50**: 1 box, 1x50 pcs)
7. One bottle of WASH BUFFER CONCENTRATE (40 mL), containing 0.2% NaN_3. *See Preparation of reagents.*

Quality certificate, Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (100, 200 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, adsorbent tissue, gamma counter.

Recommended tools and equipment

Repeating pipettes (e.g. Eppendorf or else), dispenser with at least 1.5 Litre reservoir (instead of the 2-mL pipette).

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Hemolyzed and lipemic specimens may give false values and should be avoided.

Samples with a CA72-4 concentration higher than 100 U/mL should be diluted with the Diluent (D) and re-assayed. Recommended dilution: 10-fold (450 µL D + 50 µL sample).

Preparation of reagents, storage

Store the reagents between 2-8°C after opening. At this temperature each reagent is stable until the expiration date of the kit. The actual expiration date is given on the package label and in the quality certificate.

Add the wash buffer concentrate (40 mL) to 1400 mL distilled water to obtain 1440 mL wash solution. After dilution, store at 2-8°C until the expiration date of the kit.

CAUTION! Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide , refer to Table 1.)

1. Label coated tubes in duplicate for each standard (S1-S4), control sera and samples. Label two test tubes for total counts (T).
2. Pipette 100 µL of standards, control and samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
3. Pipette 100 µL of antiserum into each tube (except T).
4. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube (min. 600 rpm).
5. Incubate tubes for 2 hours, shaking at room temperature.
6. Add 2.0 mL of diluted wash buffer to each tube. Decant the fluid of all tubes by the inversion of the rack.
7. Return the tube-rack to an upright position, and repeat the washing step two more times. In the upside down position place the rack on an absorbent paper for 2 minutes at least.
8. Pipette 200 µL of tracer into each tube.
9. Seal all tubes with a plastic foil. Incubate tubes overnight (18 to 22 hours) at 2 - 8°C.
11. Wash according to steps 6 and 7.
12. Count each tube for at least 60 seconds in a gamma counter.
13. Calculate the CA72-4 concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tubes	Total	Standard	Control	Sample
Standard		**100**		
Control			**100**	
Sample				**100**
Antiserum		**100**	**100**	**100**
Shake for 2 hours at room temperature				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Repeat the washing procedure 2 more times				
Tracer	**200**	**200**	**200**	**200**
Incubate overnight at 2 - 8°C				
Wash buffer		**2000**	**2000**	**2000**
Decant the fluid and blot on filter paper				
Repeat the washing procedure 2 more times				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.
Calculate the average count per minute (cpm) for each pair of assay tubes.
Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{1\text{-}4}/C_{I\text{-}II}/M_x\ (cpm)}{T(cpm)} \times 100$$

Using semi-logarithmic graph paper plot the B/T(%) for each standard versus the corresponding concentration of CA72-4.
Determine the CA72-4 concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.
Out of fitting programs applied for computerized data processing, spline fittings are recommended.

Table 2. Typical assay data

Tubes	Mean cpm	B/T%	CA72-4 U/mL
T	248108		
S1	751	0.3	2
S2	7683	3.1	18
S3	21467	8.7	50
S4	40768	16.4	100
CI	1935	0.8	4.7
CII	25248	10.2	59.4

Characterization of assay

Specificity

The antibodies used in this assay guarantee a measurement completely specific for CA72-4.

Limit of Detection

Based on 120 determinations, with 60 blank (diluent) and 20 low-level (< 3U/mL) samples at 3 replicates each and with 95% probability:

Limit of Detection (LoD) = 1.94 U/mL

Precision and reproducibility

Four serum pools were assayed in 20 replicates to determine intra-assay precision. To determine inter-assay precision they were measured in duplicates in 22 independent assays. Values obtained are shown below.

Intra-assay		Inter-assay	
Mean (U/mL)	CV%	Mean (U/mL)	CV%
3.4	1.06	3.27	6.32
9.83	2.12	9.65	5.78
30.66	1.89	27.98	6.94
86.41	5.03	79.01	5.77

Linearity – dilution test

Four individual serum samples were diluted 10-fold gravimetrically with diluent and measured according to kit protocol. Results:

Samples	1	2	3	4
CA72-4 U/mL	71.67	72.78	56.33	76.6
Dilution	9.76	9.76	9.78	9.76
Expected U/mL	7.34	7.45	5.76	7.85
Measured U/mL	7.81	7.67	5.64	8.01
Measured/ expected (%)	106.4	102.9	97.9	102.1

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with a known amount of CA72-4. The average per cent recovery for 5 serum samples spiked with CA72-4 at 3 levels was 101.44%, with a range of 91% to 117%.

Hook effect

No hook effect is observed for concentrations lower than 1100 U/mL.

Expected Values

It is recommended that each laboratory determine a reference range for its own patient population. Serum samples from 300 presumably healthy blood donors (150 men and 150 women) were evaluated.

Median (U/mL)	1.47
Mean (U/mL)	1.77
Standard deviation (U/mL)	1.94
Samples < 4 U/mL	285 (95.0%)

Method comparison

The CA72-4 IRMA (Y) was compared to the Fujirebio Diagnostics Inc. CA72-4 RIA (X) with the use of 68 patient samples ranging from 2 to 100 U/mL. Linear regression analysis yielded the following results:

$Y = 1.087X - 0.1828$ $R^2 = 0.9259$

Procedural notes

The non-respect of the instructions in this insert may affect results significantly.
Components from various lots or from kits of different manufacturers should not be mixed or interchanged.
Source of error! Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.
Source of error! To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

Limitations

- The CA72-4 assay should not be used as a cancer screening test.
- CA72-4 assay values greater than or equal to 4 U/mL can be found in some individuals with non-malignant conditions.
- A CA72-4 value below 4 U/mL does not indicate the absence of residual cancer.
- Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other diagnostic procedures.
- Specimens from patients who have received mouse immunoglobulin for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Serum from such individuals may produce erroneous results.

Precautions

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

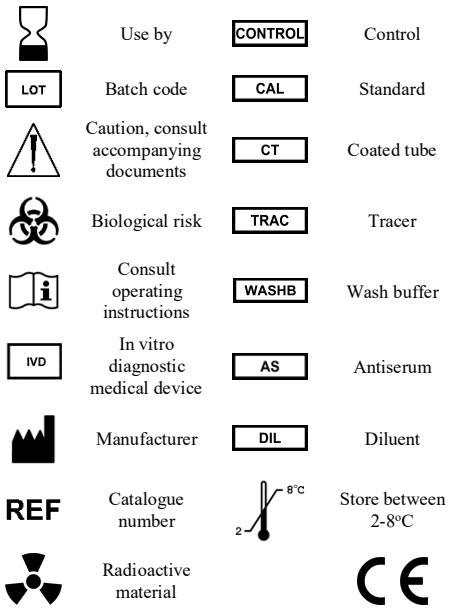
Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative for the presence of antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.
All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 123 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 60 days from availability.



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Legal note

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