Instruction for Use

【Product Name】

Cancer Antigen 72-4 (eCLIA)

【Order Information】

REF NO.	Package Size
0320501701	50 T
0320501702	2×50 T
0320501703	100 T
0320501704	2×100 T

【Intended Use】

Immunoassay for in vitro quantitative determination of Cancer Antigen 72-4 (CA72-4) in human serum and plasma, and for clinical monitoring of gastrointestinal system and other malignant tumors.

Tumor associated glycoprotein TAG72, also known as CA72-4, is a large molecular weight mucin-like protein (approximately 200-400 kD) found on the surface of a variety of cancer cells[1]. Elevated serum levels are mainly seen in patients with gastric, colorectal, and ovarian cancer[2], but are also seen in some non-malignant diseases.

For gastric cancer, the diagnostic sensitivity of CA72-4 is 33%, while the sensitivity increased to 66% when CA72-4, CEA, CA125, and CA19-9 were used in combination[3]. It has been found that the preoperative serum level of CA72-4 has the highest value for the indication of advanced disease in patients with gastric cancer[4].

For ovarian cancer, the diagnostic sensitivity of CA72-4 is usually 47-80%[5], and the sensitivity for myxoid ovarian cancer is higher than that of CA125, combined use of them can improve the sensitivity for initial diagnosis and dynamic monitoring of ovarian cancer. The diagnostic sensitivity for colorectal cancer is 20-41%[6], and the diagnostic specificity for benign colon disease is 98%.

It can be used for dynamic monitoring of the conditions of patients with confirmed malignant tumors. It cannot be used as the basis for early diagnosis or confirmed diagnosis of malignant tumors, and cannot be used for tumor screening in the general population.

【Principles of the testing method】

Sandwich principle. Total duration of assay: 9 minutes.

30 µL of sample, biotinylated CA72-4 monoclonal antibody and the ruthenium (Ru) complex-labeled CA72-4 monoclonal antibody form an antigen-antibody sandwich complex, which is bound to streptavidin coated magnetic particles, and then transferred to the measuring cell where the magnetic particles are captured onto the surface of the electrode, while the unbound substances are washed away.

Chemiluminescence is generated after the electrode is electrified, and the generated optical signal is measured by a photomultiplier, processed by an instrument, and the concentration of CA72-4 in the sample is calculated based on the calibration curve.

【Main Components】

The reagent pack consists of MB, RA, RB, calibrators and control materials (optional), and different components and reagents of lot numbers should not be used interchangeably.

Components	Ingredients	Volume (2×50T)	Volume (50T)	Volume (100T)	Volume (2×100T)
(MB)	Streptavidin coated magnetic particles, 0.45 mg/mL; 0.1 M phosphate-buffered saline (PBS); ProClin300	2×1.8 mL	1×1.8 mL	1×3.5 mL	2×3.5 mL
(RB)	Biotinylated CA72-4 antibody, 1.0 mg/L; 0.05 M HEPES; ProClin300	2×3.3 mL	1×3.3 mL	1×6.5 mL	2×6.5 mL
(RA)	Ru complex-labeled CA72-4 antibody, 2.0 mg/L; 0.05 M HEPES; ProClin300	2×3.3 mL	1×3.3 mL	1×6.5 mL	2×6.5 mL
Calibrators	CA72-4 antigen, 0.05 M PBS buffer, ProClin 300	High: 1×1.0 mL Low: 1×1.0mL			
Control Materials (Optional)	CA72-4 antigen, 0.05 M PBS buffer, ProClin 300	High: 1×1.0 mL Low: 1×1.0 mL			

Traceability: This method can be traced to Roche Elecsys CA72-4.

See the control material value sheet for the target values and ranges of control materials.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic).

Additional materials for Automated ECL Immunoassay Analyzers eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF [illegible], Assay Cup/Assay Tip, [illegible]
- REF [illegible], Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Storage and Shelf Life】

Unopened reagent kit, calibrators and control materials should be placed at 2~8ºC and will be valid for 18 months.

Once opened, they can be stored at 2~8ºC for 56 days, and reagent kit can also be stored in machine (2~15ºC).

The manufacturing date is labeled on the box, kit and bottles, and the expiration time is 18 months after production.

Damaged, expired or contaminated reagents should be discarded.

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, preparation and storage】

It is recommended to use human serum samples or plasma samples collected with EDTA-2K, EDTA-3K, heparin lithium, heparin sodium blood collection tubes.

Blood samples should be collected in accordance with the standard operation of venipuncture. After the sample is completely coagulated, centrifugation should be performed to remove residual cellular substances. The sample should be free of air bubbles during testing. Lipid layer covering the sample after centrifugation should be removed. It is not recommended to use hemolyzed samples.

Samples can be stored for 24 hours at room temperature (18~28ºC), 30 days at 2~8ºC, and 3 months at -15ºC or below. Samples should avoid repeated freeze-thaw cycles. Do not use the samples after three freeze-thaw cycles.

【Testing Procedure】

Testing procedure and precautions

Before testing, the system operation manual of the measuring instrument should be carefully read, so as to obtain relevant information such as system operating procedures, sample management, safety precautions and maintenance, and materials required for testing should be prepared. CA72-4 testing procedures should be called and set up in accordance with the system operating procedures.

Before using the reagent, put it into the analyzer 30 minutes in advance to automatically stir the magnetic bead particles and keep them in suspension.

Recommended environment temperature for testing: 10~30ºC; relative humidity: ≤80%.

Calibration

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent information should be imported into the analyzer via radio frequency identification reagent card (refer to instrument user manual).

Recalibration is recommended as follows :

(1) When using a new lot of reagent;
(2) When the same lot of reagents has been used on the analyzer for more than 28 days;
(3) If the quality control result exceeds the defined limit;
(4) When changing the buffers of different lot numbers.

Quality Control procedure

For quality control, use CA72-4 control materials.

In addition, other suitable control material can be used.

Control materials for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Please make sure that the correct values are used.

Calculation

The system software can automatically calculate the analyte concentration, with the result in U/mL.

Specimen dilution

CA72-4 concentration in the sample higher than the upper limit of detection can be diluted with sample diluent.

The recommended dilution ratio is 1:2 (automatic dilution by instrument or manual dilution). If manual dilution, the result should be multiplied by the dilution ratio. If instrument automatic dilution, the instrument will automatically calculate the result.

【Biological Reference Interval】

By analyzing the serum samples of 265 healthy human from hospitals, it was

calculated that the upper limit of the reference range of 95% is 0.90 U/mL.
Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

【Interpretation of results】

When interpreting the test results, it is necessary to refer to the patient's overall clinical situation, including: symptoms, medical history as well as other corresponding data and information.

【Limitations】

The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.

The detection range of the kit is 0.15~300 U/mL. If the CA72-4 concentration in the sample is lower than the lower limit of detection, the result is reported as <0.15 U/mL; if the CA72-4 concentration in the sample is higher than the upper limit of detection, the result is reported as >300 U/mL (the 2-fold diluted sample is reported as > 600 U/mL).

When jaundice (bilirubin) is ≤ 60 mg/dL, hemolysis (hemoglobin) is ≤ 1000 mg/dL, lipemia (triglyceride) is ≤ 1500 mg/dL, biotin is ≤ 30 ng/mL, and HAMA is ≤ 30 ng/mL in the sample, the interference deviation of the measured results is within ±10%. When the concentration of rheumatoid factor in the sample is ≤ 800 IU/mL, the relative recovery of the test results is between 90% and 110%.

When the sample contains adriamycin (100 μg/mL), methotrexate (500 μg/mL), cyclophosphamide (250 μg/mL), 5-fluorouracil (100 μg/mL), cisplatin (1000 μg/mL), aspirin (500 μg/mL) and 4-Acetamidophenol (200 μg/mL), the interference deviation of the measured results is within ±10%.

Samples of other tumor markers containing 1000 ng/mL alpha fetoprotein, 1000 U/mL cancer antigen 125, 100 U/mL cancer antigen 15-3, 1000 U/mL cancer antigen 19-9, 1000 ng/mL carcinoembryonic antigen, 500 ng/mL cytokeratin 19 fragment 21-1, 350 ng/mL neuronspecific enolase, 100 ng/mL prostate-specific antigen and 1000 ng/mL ferritin are tested, and the CA72-4 measured results are not greater than 5 U/mL.

When the CA72-4 concentration reaches 15000 U/mL, the test results are not be affected by the hook effect.

【Analytical Performance】

Limit of Detection

Limit of Blank = 0.15 U/mL.
Limit of Detection = 0.5 U/mL.

Accuracy

The relative deviation of its test results is within ±10%.

Linearity

Within the range of 0.5 U/mL~300 U/mL, the correlation coefficient (r) of the kit should not be less than 0.9900.

Within-run imprecision (repeatability)

The coefficient of variation (CV) ≤ 8.0%.

Between-lot imprecision

The coefficient of variation (CV) ≤ 10.0%.

Homogeneity of calibrators and control materials

Within-bottle homogeneity: coefficient of variation (CV) ≤ 8%.

Accuracy of calibrators

The relative deviation of its test results is within ±10%.

Accuracy of quality control

The measured results of supporting assigned quality control of the kit should be within the quality control range

Method comparison

A comparison of the Lifotronic CA72-4 assay (y) with the Roche Elecsys CA72-4 Method (x) using clinical samples gave the following correlations:
Linear regression:
y=0.9409x-0.7383
r =0.990
Number of samples measured: 120
The samples concentration were between approx 0.52 U/mL and 268.3 U/mL.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.
Exercise the normal precautions required for handling all laboratory reagents.
All reagents and samples including specimen, reagents, calibrators and control materials should avoid foaming before and during test.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.
Any serious incident that would be occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours/spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant.

【Symbols Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date		In vitro diagnostic medical device
	Batch code		Sufficient for <n> tests
	Serial number		Biological risks
	Temperature limit		This way up
	Authorized representative in the European Community		Indicates this device is in compliance with Europe Directive.
	Catalogue number	P/N	Material code
	Warning		Date of manufacture
	Unique device identifier		

【References】

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2. Guadagni F, Roselli M, Cosimelli M, et al. CA72-4 Serum Marker-A New Tool in the Management of Carcinoma Patients. Cancer Invest 1995; 13(2): 227-238.
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5. Hasholzner U, Baumgartner L, Stieber P, et al. Clinical significance of the tumor markers CA125 II and CA72-4 in ovarian carcinoma. Int J Cancer (Pred Oncol) 1996; 69(4): 329-334.
6. Guadagni F, Roselli M, Cosimelli M, et al. TAG-72 Expression and its Role in the Biological Evaluation of Human Colorectal Cancer. Anticancer Res 1996; 16: 2141-2148.

【Manufacturer】

Shenzhen Lifotronic Technology Co., Ltd.
Lifotronic Tower, No. 8, Qiuzhi East Road, Guancheng Community, Guanhu Street, Longhua, 518110 Shenzhen, PEOPLE'S REPUBLIC OF CHINA
E-mail: inter-service@lifotronic.com

【European Representative】

Shanghai International Holding Corp. GmbH (Europe)
Eiffestrasse 80, 20537 Hamburg, Germany
Tel. +49-40-2513175 Fax. +49-40-255726

【Version and Revision】

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