Elecsys CA 72-4

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REF		Σ	SYSTEM
09744525190	09744525500	100	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
CA 72-4	10225

Please note

The measured CA 72-4 value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the CA 72-4 assay method used. CA 72-4 values determined on patient samples by different testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations. If there is a change in the CA 72-4 assay procedure used while monitoring therapy, then the CA 72-4 values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Intended use

Immunoassay for the in vitro quantitative determination of CA 72-4 in human serum and plasma. The assay in particular serves as an aid in the therapeutic monitoring of carcinomas of the stomach and ovaries.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

The tumor associated glycoprotein (TAG) 72, also known as CA 72-4 is a mucin protein of high molecular weight (approximately 200-400 kD) and found on the surface of many cancer cells, including stomach, ovary, breast, colon and pancreatic cells.[1] An antibody construct directed against TAG 72 has been proposed as an anti-tumor agent against ovarian and prostate cancer.[2]

Elevated serum levels are primarily found in gastric cancer patients,[3,4] but can also be found in certain non-malignant diseases like pneumonia, pancreatitis, liver cirrhosis and ovarian cysts.[5] The most important advantage of CA 72-4 is its ability to discriminate between malignant and non-malignant gastric and ovarian diseases.[3,6]

Gastric and Ovarian Cancer:

For gastric cancer, a diagnostic sensitivity of 33 % was reported for CA 72-4.[7] Monitoring treatment and disease course in patients with gastric and ovarian cancer is the main indication for CA 72-4. After surgical intervention, CA 72-4 levels return to normal and remain within the normal range in cases where tumor tissue is no longer present.[8]

A diagnostic sensitivity of 47-76 % has been reported in ovarian carcinoma.[9] Especially for mucinous ovarian cancer, the diagnostic sensitivity of CA 72-4 is greater than that of CA 125.

The Elecsys CA 72-4 assay utilizes the following two monoclonal antibodies to detect the mucin, TAG 72:[10]

- B72.3 monoclonal antibody, which has been raised against a membrane-enriched extract of mammary carcinoma metastases[11] and
- CC49 monoclonal antibody, specific to highly-purified TAG 72.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 18 µL of sample, a biotinylated monoclonal CA 72-4-specific antibody (CC49), and a monoclonal CA 72-4-specific antibody (B72.3) labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack is labeled as CA72-4.

M Streptavidin-coated microparticles, 1 bottle, 7.2 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-CA 72-4-Ab~biotin, 1 bottle, 7.8 mL:
Biotinylated monoclonal anti-CA 72-4 antibody (CC49; mouse) 1 mg/L; phosphate buffer 100 mmol/L, pH 6.8; preservative.

R2 Anti-CA 72-4-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 7.8 mL:
Monoclonal anti-CA 72-4 antibody (B72.3; mouse) labeled with ruthenium complex 6 mg/L; phosphate buffer 100 mmol/L, pH 6.8; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
Prevention:	
P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Criterion: Slope 0.9-1.1 + coefficient of correlation ≥ 0.95.

Stable for 30 days at 2-8 °C, 24 hours at 20-25 °C, 90 days at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 09175130190, CA 72-4 CalSet, for 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against the Enzymun-Test CA 72-4 method.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

Use PreciControl Tumor Marker or other suitable controls for routine quality control procedures.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, 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.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in U/mL or kU/L).

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1130 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: Recovery ± 0.4 U/mL of intial value for samples ≤ 4 U/mL, within ± 10 % of initial value for samples > 4 to 100 U/mL, and within ± 12 % of initial value for samples > 100 U/mL.

There is no high-dose hook effect at CA 72-4 concentrations up to 15000 U/mL.

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special cancer drugs were tested. No interference with the assay was found.

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Special cancer drugs

Drug	Concentration tested µg/mL
Carboplatin	1000
Cisplatin	225
Cyclophosphamide	1000
Doxorubicin	75
Etoposide	400
5-Fluorouracil	500
Methotrexate	1000
Mitomycin	25
Taxol	5.5
Tamoxifen	50

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.5-250 U/mL (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.5 U/mL. Values above the measuring range are reported as > 250 U/mL (or up to 500 U/mL for 2-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.5 U/mL

Limit of Detection = 1.0 U/mL

Limit of Quantitation = 1.5 U/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples with CA 72-4 concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2 (automatically by the analyzers or manually). The concentration of the diluted sample must be > 110 U/mL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Please note: A sample dependent non-linearity of dilutions has been observed for samples which are outside the measuring range.

Expected values

External studies with the Elecsys CA 72-4 assay in clinical centers in Belgium, Germany, and Roche-internal studies gave the following results for a total of 635 healthy individuals:

6.9 U/mL (95 % percentile)

5.6-8.2 U/mL (95 % confidence range of the percentile)[12]

Status: Elecsys CA 72-4 multicenter evaluation; study No. B99P026, 7/2001

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human serum 1	3.79	0.051	1.3	0.112	3.0
Human serum 2	5.31	0.082	1.5	0.136	2.6
Human serum 3	5.89	0.115	2.0	0.155	2.6
Human serum 4	124	1.20	1.0	2.26	1.8
Human serum 5	203	2.75	1.4	3.88	1.9
PreciControl TM[b)]1	4.91	0.078	1.6	0.113	2.3
PreciControl TM2	54.3	0.555	1.0	1.10	2.0

b) TM = Tumor Marker

Method comparison

a) A comparison of the Elecsys CA 72-4 assay, REF 09005706190 (**cobas e** 801 analyzer; y) with the Elecsys CA 72-4 assay, REF 07324910190 (**cobas e** 801 analyzer; x) gave the following correlations (U/mL):

Number of serum samples measured: 141

Passing/Bablok[13]	Linear regression
y = 0.945x + 0.0564	y = 0.936x + 0.223
τ = 0.963	r = 0.996

The sample concentrations were between 0.766 and 223 U/mL.

b) A comparison of the Elecsys CA 72-4 assay, REF 09005706190 (**cobas e** 402 analyzer; y) with the Elecsys CA 72-4 assay, REF 09005706190 (**cobas e** 801 analyzer; x) gave the following correlations (U/mL):

Number of serum samples measured: 143

Passing/Bablok[13]	Linear regression
y = 1.067x + 0.0262	y = 1.047x + 0.220
τ = 0.972	r = 0.999

The sample concentrations were between 0.531 and 229 U/mL.

Analytical specificity

The Elecsys CA 72-4 tumor marker assay is based on the monoclonal B72.3 and CC49 antibodies which are only available from Fujirebio Diagnostics, its licensees and its representatives. The performance characteristics of testing procedures using these antibodies cannot be assumed for test methods using other antibodies.

References

1 Muraro R, Kuroki M, Wunderlich D, et al. Generation and characterization of B72.3 2nd generation monoclonal antibodies reactive with the tumor-associated glycoprotein 72 antigen. Cancer Res 1988;48(16):4588-4596.

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2 Scott MA, Akhurst T, Lee F-T, et al. Phase I safety and biodistribution study of 124I-PEG-AVP0458 diabody in patients with TAG-72 positive ovarian and prostate cancer. In: Proceedings of the 106th Annual Meeting of the American Association for Cancer Research; 2015 Apr 18-22; Philadelphia, PA. Philadelphia (PA): AACR; Cancer Res 2015;75(15):Abstract nr CT238.

3 Guadagni F, Roselli M, Cosimelli M, et al. CA 72-4 Serum Marker - A New Tool in the Management of Carcinoma Patients. Cancer Invest 1995;13(2):227-238.

4 Filella X, Fuster J, Molina R, et al. TAG-72, CA 19-9 and CEA as tumor markers in gastric cancer. Acta Oncologica 1994;33(7):747-751.

5 Filella X, Molina R, Jo J, et al. Tumor associated glycoprotein 72 (TAG 72) levels in patients with non-malignant and malignant disease. Bull Cancer 1992;79:271-277.

6 Heptner G, Domschke S, Domschke W. Comparison of CA 72-4 with CA 19-9 and Carcinoembryonic Antigen in the Serodiagnosis of Gastrointestinal Malignancies. Scand J Gastroenterol 1989;24:745-750.

7 Yang A-P, Liu J, Lei H-Y, et al. CA 72-4 combined with CEA, CA-125 and CA 19-9 improves the sensitivity for the early diagnosis of gastric cancer. Clin Chim Acta 2014;437:183-86.

8 Reiter W, Stieber P, Reuter C, et al. Prognostic Value of Preoperative Serum Levels of CEA, CA 19-9 and 72-4 in Gastric Carcimoma. Anticancer Res 1997;17:2903-2907.

9 Hasholzner U, Baumgartner L, Stieber P, et al. Clinical significance of the tumor markers CA 125 II and CA 72-4 in ovarian carcinoma. Int J Cancer (Pred Oncol) 1996;69(4):329-334.

10 Johnson VG, Schlom J, Paterson AJ, et al. Analysis of a human tumor-associated glycoprotein (TAG-72) identified by monoclonal antibody B72.3. Cancer Res 1986;46:850-857.

11 Colcher D, Horan Hand P, Nuti M, et al. A Spectrum of monoclonal antibodies reactive with human mammary tumor cells. Proc Natl Acad Sci 1981;78(5):3199-3208.

12 Hahn GJ, Meeker WQ. Statistical Intervals: A Guide for Practitioners. John Wiley & Sons, Inc. New York 1991.

13 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate user guide or operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

FUJIREBIO Diagnostics, Inc.

CA 72-4 antibodies used inside the Roche CA 72-4 products are licensed by Fujirebio Diagnostics, Inc.

Any serious incident that has 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 Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

Rx only For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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