LDN Labor Diagnostika Nord GmbH & Co. KG
Am Eichenhain 1, 48531 Nordhorn
Telefon: +49-5921-8197 0
Telefax: +49-5921-8197 222
e-mail: info@ldn.de
Internet: http://www.ldn.de

Instructions for use

CA 19-9 ELISA

REF TM E-4500

IVD CE

CA19.9 ELISA

Introduction

Intended Use

The TM-CA19.9 ELISA is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of CA 19-9 in serum and plasma

Summary and Explanation

The sialyl Lewis[a] epitope is also known as the cancer associated antigen CA 19-9.
CA 19-9 levels are frequently elevated in the serum of patients with cancers of the pancreato-biliary system (i.e. pancreas, gallbladder, biliary tract). In addition, elevated levels of CA 19-9 have been observed in other malignancies such as lung cancer, other gastrointestinal cancers and in some nonmalignant disorders.
It must be taken into acount that patients, genotypically negative for the Lewis blood group antigen, will be unable to produce the CA 19-9 antigen even in the presence of malignant tissue.

PRINCIPLE of the test

The TM-CA19.9 ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.
The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site of the CA 19-9 molecule. An aliquot of patient sample containing endogenous CA 19-9 is incubated in the coated well with assay buffer.
After a washing step a second incubation follows with enzyme conjugate, which is an anti-CA 19-9 antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase is proportional to the concentration of CA 19-9 in the sample.
Having added the substrate solution, the intensity of colour developed is proportional to the concentration of CA 19-9 in the patient sample.

Warnings and Precautions

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request.

Reagents

Reagents provided

96 **TM E-4531 Microtiterwells**

12x8 (break apart) strips, 96 wells; Wells coated with anti-CA 19-9 antibody (monoclonal).

Standards

	Cat. no.	Standard	Concentration	Volume/Vial
CAL 0	**TM E-4501**	Zero Standard	0 U/mL	3 ml
CAL 1	**TM E-4501**	Standard 1	15 U/mL	0,5 ml
CAL 2	**TM E-4501**	Standard 2	30 U/mL	0,5 ml
CAL 3	**TM E-4501**	Standard 3	60 U/mL	0,5 ml
CAL 4	**TM E-4501**	Standard 4	120 U/mL	0,5 ml
CAL 5	**TM E-4501**	Standard 5	240 U/mL	0,5 ml

Contain non-mercury preservative.

CONTROL 1 **+** CONTROL 2 **TM E-4551 + TM E-4552 Control Low & High**

2 vials, (lyophilized), 0.5 mL each; see „Reagent Preparation"; For control values and ranges please refer to vial label or QC-Datasheet. Contain non-mercury preservative.

ASSAY-BUFF **TM E-4513 Assay Buffer**

1 vial, 7 mL, ready to use, Contains non-mercury preservative.

CONJUGATE **TM E-4540 Enzyme Conjugate**

1 vial, 14 mL, ready to use, Anti-CA 19-9 antibody conjugated to horseradish peroxidase;
Contains non-mercury preservative.

SUBSTRATE **FR E-0055 Substrate Solution**

1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).

STOP-SOLN **FR E-0080 Stop Solution**

1 vial, 14 mL, ready to use, contains 0.5M H_2SO_4,
Avoid contact with the stop solution. It may cause skin irritations and burns.

WASH- CONC 40x **FR E-0030 Wash Solution**

1 vial, 30 mL (40X concentrated), see „Preparation of Reagents".

Note: Additional *Zero Standard* for sample dilution is available upon request.

Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two months if stored as described above.

Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Control
Reconstitute the lyophilized content with 0.5 mL distilled water and let stand for 10 minutes in minimum. Mix the controls several times before use.
Note: *The reconstituted controls should be apportioned and stored at –20°C.*

Wash Solution
Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL distilled water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

Damaged Test Kits

In case of any severe damage to the test kit or components, the supplier has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

SPECIMEN Collection and Preparation

Serum or plasma (EDTA or heparin plasma) can be used in this assay.
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.
(E.g. for EDTA plasma Sarstedt Monovette – red cap - # 02.166.001;
for Heparin plasma Sarstedt Monovette – orange cap - # 02.165.001;

Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying.
Specimens held for a longer time (up to two months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Zero Standard* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) dilution 1:10: 10 µL Serum + 90 µL *Zero Standard* (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Zero Standard* (mix thoroughly).

Assay procedure

General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Test Procedure

Each run must include a standard curve.

1.	Secure the desired number of Microtiter wells in the frame holder.
2.	Dispense **50 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3.	Dispense **50 µL *Assay Buffer*** into each well.
4.	Incubate for **60 minutes** at room temperature.
5.	Briskly shake out the contents of the wells. Rinse the wells **4 times** with diluted *Wash Solution* (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets. **Important note:** The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6.	Dispense **100 µL *Enzyme Conjugate*** into each well.
7.	Incubate for **60 minutes** at room temperature.
8.	Briskly shake out the contents of the wells. Rinse the wells **4 times** with diluted *Wash Solution* (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9.	Add **100 µL** of ***Substrate Solution*** to each well.
10.	Incubate for **30 minutes** at room temperature.
11.	Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
12.	Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 240 U/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 U/mL)	0.04
Standard 1 (15 U/mL)	0.26
Standard 2 (30 U/mL)	0.44
Standard 3 (60 U/mL)	0.78
Standard 4 (120 U/mL)	1.30
Standard 5 (240 U/mL)	1.99

Expected Normal VAlues

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the CA19-9 ELISA the following values are observed:

Population	Valid N	5th Percentile (U/mL)	95th Percentile (U/mL)
Males	32	0.69	20.84
Females	39	1.30	13.38

Several studies recommended a cut-off value of 37 U/mL to differentiate between invasive and benign intraductal papillary mucinous neoplasm (IPMN) in chronic pancreatitis (10,12,13). Other studies suggest a value of 70-75 U/mL for differentiating benign from malignant pathology (14,15).

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit.
The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor.

Performance Characteristics

Assay Dynamic Range

The range of the assay is between 0.2 – 240 U/mL.

Specificity of Antibodies (Cross Reactivity)

Cross-reactivities of the assay are not known.

Sensitivity

The analytical sensitivity of the CA 19-9 ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Zero Standard and was found to be 0.20 U/mL.

Reproducibility

Intra Assay

The within assay variability is shown below:

Sample	n	Mean (U/mL)	CV (%)
1	20	27.68	8.44
2	20	47.68	9.24
3	20	74.04	9.63

Inter Assay

The between assay variability is shown below:

Sample	n	Mean (U/mL)	CV (%)
1	6	6.02	8.10
2	6	10.42	1.86
3	6	14.98	8.69

Recovery

Samples have been spiked by adding CA 19-9 solutions with known concentrations in a 1:1 ratio.
The % recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100 (expected value = (endogenous CA 19-9 + added CA 19-9) / 2; because of a 1:2 dilution of serum with spike material).

		Sample 1	Sample 2	Sample 3
Concentration [U/mL]		22.6	22.1	17.6
Average Recovery		91.9	87.7	91.1
Range of Recovery [%]	from	87.4	85.5	87.2
	to	95.9	91.6	98.5

Linearity

		Sample 1	Sample 2	Sample 3
Concentration [U/mL]		82.7	25.8	41.7
Average Recovery		100.8	109.7	102.6
Range of Recovery [%]	from	93.1	103.9	97.8
	to	108.5	114.7	104.6

Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.125 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.
The assay contains reagents to minimize interference of HAMA and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of CA 19-9 in a sample.

High-Dose-Hook Effect

No hook effect was observed in this test up to 3840.0 U/mL of CA 19-9.

Legal Aspects

Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact us.

Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point "Reliability of Results". Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point "Therapeutic Consequences" are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!