

Instructions for use
CA 19-9 ELISA

Please use only the valid version of the Instructions for Use provided with the kit

REF**TM E-4500R**

96

RUO

For research
use only –
Not for use
in diagnostic
procedures

CA19-9 ELISA

1. INTRODUCTION

1.1 Intended Use

The CA 19-9 ELISA is an enzyme immunoassay for the quantitative measurement of CA 19-9 in serum or plasma (EDTA, heparin or citrate plasma)

2. PRICIPLE OF THE TEST

The CA 19-9 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 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 sample.

WARNINGS AND PRECAUTIONS

1. This kit is for research 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 °C - 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the 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 H₂SO₄. It may cause skin irritation and burns.
18. Some reagents may 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 Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

4. REAGENTS

4.1 Reagents provided







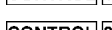

TM E-4531

 96

Microtiterwells

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

Standards and Controls -Standards: ready to use
contain non-mercury preservative

Cat. no.	Symbol	Standard	Concentration	Volume/Vial
TM E-4501		Zero Standard	0 U/ml	3 ml
TM E-4502		Standard 1	15 U/ml	0.5 ml
TM E-4503		Standard 2	30 U/ml	0.5 ml
TM E-4504		Standard 3	60 U/ml	0.5 ml
TM E-4505		Standard 4	120 U/ml	0.5 ml
TM E-4506		Standard 5	240 U/ml	0.5 ml
TM E-4551		Control low	For control values and ranges please refer to vial label or QC Report!	0.5 ml (lyophilized)*
TM E-4552		Control high		0.5 ml (lyophilized)*

* see „Reagent Preparation“

TM E-4513  **Assay Buffer** - Ready to use

Content: Contains non-mercury preservative.

Volume: 1 x 7 ml

TM E-4540  **Enzyme Conjugate** - Ready to use

Content: Anti-CA 19-9 antibody conjugated to horseradish peroxidase
Contains non-mercury preservative.

Volume: 1 x 14 ml

FR E-0055  **Substrate Solution** - Ready to use

Content: Tetramethylbenzidine (TMB)

Volume: 1 x 14 ml

FR E-0080  **Stop Solution** - Ready to use

Content: contains 0.5 M H₂SO₄

Volume: 1 x 14 ml

Avoid contact with Stop Solution. It may cause skin irritations and burns.

Hazard identification:



H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

FR E-0030  **Wash Solution** - 40x concentrated

Volume: 1 x 30 ml

see "Reagent Preparation"

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

4.2 Materials required but not provided

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

4.3 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 8 weeks if stored as described above.

4.4 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.

4.5 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 Safety Data Sheet.

4.6 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.

5. SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA, heparin or citrate 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.

5.1 Specimen Collection

Serum:

Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

5.2 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.

5.3 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).

6. ASSAY PROCEDURE

6.1 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.

6.2 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 <u>with new disposable tips</u> 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 <i>Wash Solution</i> (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 <i>Wash Solution</i> (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 <i>Stop Solution</i> .

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Manual method: Using linear 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 Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) 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.

6.3.1 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

7. 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	n	Mean (U/ml)	Median (U/ml)	5 th - 95 th Percentile (U/ml)	2.5 th - 97.5 th Percentile (U/ml)	Range (min. - max.) (U/ml)
Males and females	71	8.08	5.70	1.15 - 20.90	0.27 - 26.15	0.04 - 28.70

8. QUALITY CONTROL

Good laboratory practice requires that controls be run with each standard 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 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.

9. PERFORMANCE CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.26 U/ml – 240 U/ml.

9.2 Specificity of Antibodies (Cross Reactivity)

Cross-reactivities of the assay are not known.

9.3 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 0.26 U/ml.

9.4 Reproducibility

9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (U/ml)	CV (%)
1	20	27.7	8.4
2	20	47.7	9.2
3	20	74.0	9.6

9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (U/ml)	CV (%)
1	39	5.7	12.2
2	40	10.7	8.2
3	40	11.4	10.5

9.4.3 Inter-Lot

The inter-assay (between-lots) variation was determined by measuring each sample 6 times with 3 different kit lots (n = 18):

Sample	n	Mean (U/ml)	CV (%)
1	18	10.5	10.1
2	18	14.3	7.8
3	18	25.2	3.4
4	18	28.6	1.0

9.5 Recovery

Samples have been spiked by adding CA 19-9 solutions with known concentrations.

The % recovery was calculated by multiplying the ratio of the measured and expected values with 100.

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

9.6 Linearity

Samples were measured undiluted and in serial dilutions from 1:2 to 1:16 with Zero Standard. The recovery (%) was calculated by multiplying the ratio of expected and measured values with 100.

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

10. 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.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 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.

10.2 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.







10.3 High-Dose-Hook Effect

Hook effect was not observed in this test up to a concentration of 15 360 U/ml of CA 19-9.

11. REFERENCES / LITERATURE

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Symbols:

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