



User's Manual

Human CA19-9 ELISA Kit



DEIA514



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

 Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

 Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe)  Fax: 1-631-938-8221

 Email: info@creative-diagnostics.com  Web: www.creative-diagnostics.com

PRODUCT INFORMATION

Intended Use

The CA 19-9 ELISA is an enzyme immunoassay for measurement of CA 19-9 in serum and plasma.

Principles of Testing

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.

Reagents And Materials Provided

1. Microtiterwells, 12x8 (break apart) strips, 96 wells; Wells coated with anti-CA 19-9 antibody (monoclonal).
2. Zero Standard, 1 vial, 3 mL, ready to use; Contains non-mercury preservative.
3. Standard (Standard 1-5), 5 vials, 0.5 mL, ready to use; Concentrations: 15 – 30 – 60 – 120 - 240 U/mL; Contain non-mercury preservative.
4. Control Low & High, 2 vials, (lyophilized), 0.5 mL each; Contain non-mercury preservative.
5. Assay Buffer, 1 vial, 7 mL, ready to use; Contains non-mercury preservative.
6. Enzyme Conjugate, 1 vial, 14 mL, ready to use, Anti-CA 19-9 antibody conjugated to horseradish peroxidase; Contains non-mercury preservative.
7. Substrate Solution, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
8. Stop Solution, 1 vial, 14 mL, ready to use, contains 0.5M H₂SO₄, Avoid contact with the stop solution. It may cause skin irritations and burns.
9. Wash Solution, 1 vial, 30 mL (40X concentrated).

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

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450 ± 10 nm)
2. Calibrated variable precision micropipettes.
3. Absorbent paper.
4. Distilled or deionized water

5. Timer
6. Graph paper or software for data reduction

Storage

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.

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, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.

Plasma:

Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.

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. If EDTA plasma is stored at 2 °C to 8 °C, it must be assayed within 48 hours. 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).

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.

Assay Procedure

General Remarks

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
4. 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.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Assay 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 $450 \pm 10 \text{ nm}$ with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

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.

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 CD directly.

Calculation

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 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.

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

Precautions

1. 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.
2. 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.
3. 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.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. 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.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. 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 samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. 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.
16. Avoid contact with Stop Solution containing 0.5 M H₂SO₄. It may cause skin irritation and burns.
17. Some reagents may contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
18. 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.
19. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
20. 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 directly from CD.

Limitations

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.