

3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

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Disclaimer

These ELISA Kits have not been tested for clinical use and are not approved in the United States by the FDA for diagnostic clinical use. They are components or reagents made solely for research use, further manufacturing and export use. It is the commitment of the buyer to receive its products solely for the purpose of exportation or research, and not for the purposes of clinical diagnostic use.

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CA19-9 ELISA

Catalog No. IB19124 (96 Tests)

INTENDED USE

The CA19-9 ELISA Kit is intended for the determination of the Cancer Antigen CA19-9 concentration in human serum.

SUMMARY AND EXPLANATION

A group of mucin type glycoprotein Sialosyl Lewis Antigens (SLA), such as CA19-9 and CA19-5, have come to be recognized as circulating cancer associated antigens for gastrointestinal cancer. CA19-9 represents the most important and basic carbohydrate tumor marker. The immunohistologic distribution of CA19-9 in tissues is consistent with the quantitative determination of higher CA19-9 concentrations in cancer than in normal or inflamed tissues. Recently reports indicates that the serum CA19-9 level is frequently elevated in the serum of subjects with various gastrointestinal malignancies, such as pancreatic, colorectal, gastric and hepatic carcinomas. Together with CEA, elevated CA19-9 is suggestive of gallbladder neoplasm in the setting of inflammatory gallbladder disease. This tumor-associated antigen may also be elevated in some non-malignant conditions. Research studies demonstrate that serum CA 19-9 values may have utility in monitoring subjects with the above-mentioned diagnosed malignancies. It has been shown that a persistent elevation in serum CA19-9 value following treatment may be indicative of occult metastatic and/or residual disease. A persistently rising serum CA 19-9 value may be associated with progressive malignant disease and poor therapeutic response. A declining CA 19-9 value may be indicative of a favorable prognosis and good response to treatment.

PRINCIPLE OF THE TEST

The CA19-9 ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact CA19-9 molecule is used for solid phase immobilization (on the microtiter wells). Another CA 19-9 monoclonal antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react sequentially with the two antibodies, resulting in the CA19-9 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After two separate incubation steps at 37°C for 90 minutes, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of CA19-9 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

MATERIALS PROVIDED	96 Tests
1. Microwells coated with CA 19-9 MAb	12x8x1
2. CA 19-9 Standard set: 6 vials (ready to use)	0.5ml
3. CA 19-9 Assay buffer	13 ml
4. Enzyme Conjugate Concentrate (12x)	1.1ml
5. CA 19-9 Conjugate Diluent	13ml
6. Wash Buffer concentrate (20X)	50ml
7. TMB Reagent	11ml
8. Stop Solution: 1 bottle (ready to use)	11ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. precision pipettes and tips
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This test kit is designed for Research Use Only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

REAGENT PREPARATION

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50ml of Wash buffer (20x) into distilled water to prepare 1000ml of Wash buffer (1x). Wash Buffer is stable for 1 month at 2-8 C. Mix well before use.
2. To prepare Working CA 19-9 Conjugate Reagent:
 - a. For 3.0 ml, which is more than enough for 24 wells: Add 0.25 ml of Conjugate Concentrate (12x) to 2.75 ml of the Enzyme Conjugate Diluent (1:11 dilution) and mix well.
 - b. For 6.0 ml, which is more than enough for 48 wells: Add 5.5 ml of Conjugate Concentrate (12x) to 5.5 ml of the Enzyme Conjugate Diluent (1:11 dilution) and mix well.
 - c. For 9.0 ml, which is more than enough for 72 wells: Add 0.75ml of Conjugate Concentrate (12x) to 8.25ml of the Enzyme Conjugate Diluent (1:11 dilution) and mix well.
 - d. For 12.0 ml, which is more than enough for 96 wells: Add 1.0 ml of Conjugate Concentrate (12x) to 11.0 ml of the Enzyme Conjugate Diluent (1:11 dilution) and mix well.
3. The Working CA 19-9 Conjugate Reagent needs to be prepared freshly every time before use.
4. The amount of conjugate diluted depends on your assay size. Discard the excess after use.

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (18-26 °C) and gently mix.

1. Secure the desired number of coated wells in the holder.
2. Dispense 10 µl of CA19-9 standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl of CA 19-9 Assay Buffer (green-color solution) into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix them completely.
5. Incubate at 37°C for 90 minutes.
6. Remove the incubation mixture by emptying the plate content into a waste container.

7. Rinse and empty the microtiter plate 4 times with Wash buffer (1X) and then one time with distilled or deionized water. (Please do not use tap water.)
8. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of the Working Conjugate Reagent (red-colored solution) into each well. Mix gently for 30 seconds.
10. Incubate at 37°C for 90 minutes.
11. Remove the incubation mixture by emptying the plate content into a waste container.
12. Rinse and empty the microtiter plate 4 times with Wash buffer (1X) and then one time with distilled or deionized water. (Please do not use tap water.)
13. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
14. Dispense 100 µl of the TMB Reagent into each well. Gently mix for 10 seconds.
15. Incubate at room temperature in the dark for 20 minutes without shaking.
16. Stop the reaction by adding 100 µl of Stop Solution to each well.
17. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
18. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

CALCULATIONS AND RESULTS

1. Calculate the average absorbance values (A450) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml via best fit quadratic on linear graph paper, with absorbance 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 of CA19-9 in U/ml from the standard curve.
- 4.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against CA19-9 concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve in each experiment.

CA19-9 (U/ml)	Absorbance (450 nm)
0	0.139
25	0.323
75	0.604
150	0.884
300	1.487
600	2.713

EXPECTED VALUES AND SENSITIVITY

Healthy men and women are expected to have CA19-9 assay values below 35 U/ml. The minimum detectable concentration of CA19-9 in this assay is estimated to be 10 U/ml.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.