

bi-x-act [®] of FINLAND	CA199 EIA TEST KIT (Gastrointestinal Cancer Antigen)
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**Enzyme Immunoassay for the quantitative determination of Gastrointestinal Cancer
Antigen CA19-9 in Human Serum**

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

INTENDED USE

For the quantitative determination of the Cancer Antigen CA19-9 concentration in human serum.

INTRODUCTION

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.

REAGENTS

Materials provided with the kit:

- Antibody-Coated Wells (1 plate, 96 wells)
Microtiter wells coated with CA19-9 MoAb
- Reference Standard Set (1.0 ml/vial)
Contains 0, 25, 75, 150, 300, and 600 Unit/ml of CA19-9 in bovine serum with preservatives; liquid, ready to use
- CA19-9 Enzyme Conjugate Concentrate (12x), 1.1 ml
Contains CA19-9 MoAb conjugated to horseradish peroxidase with preservatives
- CA19-9 Conjugate Diluent, 13 ml
Contains bovine serum, tris buffer and preservatives
- CA 19-9 Assay Buffer, 13 ml
Phosphate buffer with proteins and preservatives
- Wash Buffer Concentrate (20x)
Contains phosphate buffer and Tween 20
- TMB Reagent (11 ml)
Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution
- Stop Solution -1N HCl (11 ml)
Diluted hydrochloric acid

Materials required but not provided:

- Precision pipettes and tips: 10 µl and 100 µl.
- Distilled water.
- Vortex mixer
- Absorbent paper or paper towel
- Graph paper
- A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at a wavelength of 450 nm

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

3. To prepare **Working CA 19-9 Conjugate Reagent**:

- For 3.0 ml, which is more than enough for 24 wells: Add 0.25 ml of Conjugate Concentrate (12x) to 2.75ml of the Enzyme Conjugate Diluent (1:11 dilution) and mix well.
- For 6.0 ml, which is more than enough for 48 wells: Add 0.5 ml of Conjugate Concentrate (12x) to 5.5 ml of the Enzyme Conjugate Diluent (1:11 dilution) and mix well.
- For 9.0 ml, which is more than enough for 72 wells: Add 0.75 ml of Conjugate Concentrate (12x) to 8.25 ml of the Enzyme Conjugate Diluent (1:11 dilution) and mix well.
- 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.

The Working CA 19-9 Conjugate Reagent needs to be prepared freshly every time before use.

The amount of conjugate diluted depends on your assay size. Discard the excess after use.

ASSAY PROCEDURE

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 flick the microtiter wells 4 times with diluted 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 flick the microtiter wells 4 times with diluted 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.**

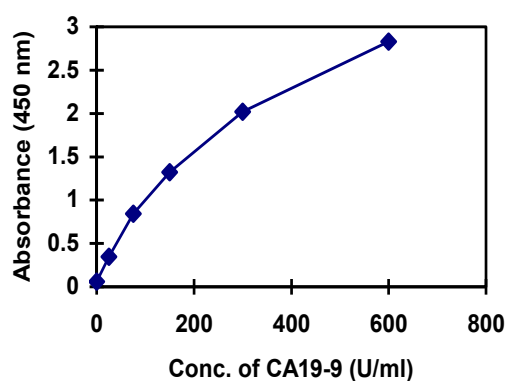
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) 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.

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	0.058
25	0.347
75	0.845
150	1.322
300	2.020
600	2.830



EXPECTED VALUES

Healthy men and women are expected to have CA19-9 assay values below 35 U/ml.

PERFORMANCE CHARACTERISTICS

1. Accuracy

A statistical study using patient samples demonstrated good correlation of results with the commercially available kits as shown below:

Comparisons between CA199 ELISA Test and Bayer Centaur CA 19-9 kits provide the following data:

N = 56
 Correlation coefficient = 0.921
 Slope = 4.207
 Intercept = -55.27
 CA199 ELISA Test Mean = 63 U/mL
 Bayer Centaur Mean = 209 U/mL

2. Sensitivity

The minimal detectable concentration of CA 19-9 by this assay is estimated to be 5 U/mL.

3. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of four different control sera in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3	4
Number of Replicates	24	24	24	24
Mean CA 19-9 (U/mL)	15	76	257	476
Standard Deviation	0.3	2.0	6.7	32.5
Coefficient of Variation (%)	2.2	2.7	2.6	6.8

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of four different control sera in several different assays. Between-assay variability is shown below:

Serum Sample	1	2	3	4
Number of Replicates	20	20	20	20
Mean CA 19-9 (U/mL)	14	75	258	513
Standard Deviation	0.9	3.4	12.4	26.5
Coefficient of Variation (%)	6.4	4.5	4.8	5.2

4. Recovery and Linearity Studies

a. Recovery

Various patient serum samples of known CA 19-9 levels were mixed and assayed in duplicate. The average recovery was 97.1%.

	Expected Concentration (U/ml)	Observed Concentration (U/ml)	% Recovery
1	53	53	98.5
.	161	153	94.8
2	159	146	91.9
.	482	499	103.4
3			
.			
4			
.			
Average Recovery = 97.1%			

b. Linearity

Four patient samples were serially diluted with the zero standard in a linearity study. The average recovery was 100.7%.

#	Dilution	Expected Conc. (U/mL)	Observed Conc. (U/mL)	% Recovery
1.	Undiluted	----	382.56	----
	1:2	196.34	191.30	97.4
	1:4	98.56	95.60	97.0
	1:8	46.32	47.80	103.2
	1:16	23.87	23.90	100.1
Mean = 99.4 %				
2.	Undiluted	----	276.70	----
	1:2	139.89	138.40	98.9
	1:4	67.27	69.20	102.9
	1:8	35.66	34.60	97.0
	1:16	16.80	17.30	103.0
Mean = 100.5 %				
3.	Undiluted	----	332.16	----
	1:2	164.06	166.10	101.2
	1:4	85.76	83.00	96.8
	1:8	46.56	41.50	89.1
	1:16	22.18	20.80	93.8
Mean = 95.2%				
4.	Undiluted	----	123.30	----
	1:2	53.56	61.70	115.2
	1:4	29.03	30.80	106.1
	1:8	14.30	15.40	107.7
	1:16	7.58	7.70	101.6
Mean = 107.6%				

5. Specificity

The following substances were tested for cross-reactivity:

Analyte Tested	Concentration	Produced Intensity Equivalent To CA 19-9 (U/mL)
PSA	1,000 ng/mL	0
	2,500 ng/mL	0
	5,000 ng/mL	0
CEA	100 ng/mL	0
	250 ng/mL	6
	500 ng/mL	18
CA 125	500 ng/mL	5
	1,000 ng/mL	17
	2,500 ng/mL	34
CA 15-3	250 ng/mL	0
	500 ng/mL	0
	1,000 ng/mL	2.6
	2,000 ng/mL	8.7
PAP	6,000 ng/mL	0
	12,000 ng/mL	0
AFP	5,000 ng/mL	0
	10,000 ng/mL	0
	25,000 ng/mL	0
	50,000 ng/mL	0

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.
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.

REFERENCES

- ¹ Glenn, J., Steinberg, W.M., Kurtzman, S.H., et al., Evaluation of the utility of a radioimmunoassay for serum CA 19-9 levels in patients before and after treatment of carcinoma of the pancreas. *J. Clin. Oncol.*, 1988; 6: 462-468.
- ² Hayakawa, T., Kondo, T., Shibata, T., et al., Sensitive serum markers for detecting pancreatic cancer. *Cancer*, 1988; 61: 1827-1831.
- ³ Koprowski, H., Herlyn, M., Steplewski, Z., et al. Specific antigen in serum of patients with colon carcinoma. *Science*, 1981; 212: 53-55.
- ⁴ Malesci, A., Tommasini, M.A., Bonato, C., Determination of CA 19-9 antigen in serum and pancreatic juice for differential diagnosis of pancreatic adenocarcinoma from chronic pancreatitis. *Gastroenterology*, 1987; 92: 60-67.

- ⁵ Safi, F., Roscher, R., Bittner, R., et al., High sensitivity and specificity of CA 19-9 for pancreatic carcinoma in comparison to chronic pancreatitis. Serological and immunohistochemical findings. *Pancreas*, 1987; 2: 398-403.
- ⁶ Steinberg, W., The clinical utility of the CA 19-9 tumor-associated antigen. *Am. J. Gastroenterology*, 1990; 85: 350-355.
- ⁷ Steinberg, W.M., Gelfand, R., Anderson, K.K., et al., Comparison of the sensitivity and specificity of the CA 19-9 and carcinoembryonic antigen assays in detecting cancer of the pancreas. *Gastroenterology*, 1986; 90: 343-349.
- ⁸ Takasaki, H., Uchida, E., Tempero, M.A., et al., Correlative study on expression of CA 19-9 and DU-PAN-2 in tumor tissue and in serum of pancreatic cancer patients. *Cancer Research*, 1988; 48: 1435-1438.
- ⁹ Tatsuta, M., Yamamura, H., Iishi H., et al., Values of CA19-9 in the serum, pure pancreatic juice, and aspirated pancreatic material in the diagnosis of malignant pancreatic tumor. *Cancer*, 1985; 56: 2669-2673.
- ¹⁰ Wang, T-H., Lin, J-W., Chen, D-S., et al., Noninvasive diagnosis of advanced pancreatic cancer by real-time ultrasonography, carcinoembryonic antigen, and carbohydrate antigen 19-9. *Pancreas*, 1986; 1: 219-223.
- ¹¹ Strom, B.L., Maislin G.M., West, S.L., et al., Serum CEA and CA19-9: potential future diagnostic or screening tests for gallbladder cancer? *Int. J. Cancer*, 1990; 45: 821-824.