

**CANCER ANTIGEN CA19-9  
ENZYME IMMUNOASSAY  
TEST KIT**

Catalog Number: BC-1017

**Enzyme Immunoassay for the Quantitative Determination of Gastrointestinal Cancer Antigen CA 19-9 in Human Serum**

**FOR IN VITRO DIAGNOSTIC USE ONLY**

Store at 2 to 8°C.

**PROPRIETARY AND COMMON NAMES**

CA19-9 Enzyme Immunoassay

**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). A goat anti-CA19-9 antibody conjugated to horseradish peroxidase (HRPO) 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 test kits:**

- Murine monoclonal anti-CA19-9 coated 96 well microtiter plate.
- Assay Buffer; 13 ml.
- Enzyme Conjugate Reagent, 22 ml.
- CA19-9 reference standards, containing 0, 15, 30, 60, 120, and 240 U/ml CA19-9, liquid. Ready for use. 1 set.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

**Materials required but not provided:**

- Precision pipettes and tips, 0.1ml, 0.2ml, 1.0ml.
- Distilled water.
- Vortex mixer
- Absorbent paper or paper towel
- Graph paper
- A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at a wavelength of 450nm

**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 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

#### REAGENT PREPARATION

All reagents should be brought to room temperature (18-25°C) before use.

#### ASSAY PROCEDURE

- Secure the desired number of coated wells in the holder.
- Dispense 100µL of CA19-9 standards, specimens, and controls into appropriate wells.
- Dispense 100µL of Assay Buffer (green-colored solution) to each well. Mix gently for 30 seconds.
- Incubate at 37°C for 90 minutes.
- Remove the incubation mixture by emptying the plate content into a waste container.
- Rinse and empty the microtiter plate 5 times with distilled or deionized water. (Please do not use tap water.)
- Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
- Dispense 200µL of Enzyme Conjugate Reagent (red-colored solution) into each well. Mix well.
- Incubate at 37°C for another 90 minutes.
- At the end of the 90-minute incubation, remove the contents and wash the wells as described in steps 6-7 above.
- Dispense 100µL of the TMB Reagent into each well. Gently mix for 10 seconds.
- Incubate at room temperature in the dark for 20 minutes without shaking.
- Stop the reaction by adding 100µl of Stop Solution to each well.
- Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
- Read the optical density at 450nm with a microtiter plate reader **within 15 minutes.**

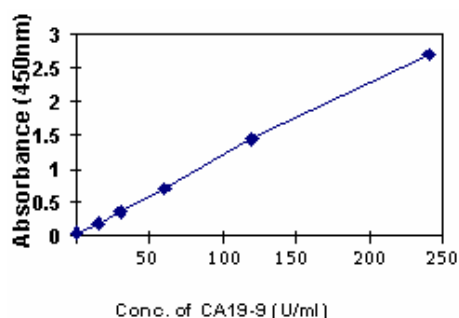
#### CALCULATION OF RESULTS

- Calculate the average absorbance values (A<sub>450</sub>) for each set of reference standards, control, and samples.
- Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
- 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 Values (U/ml)	Absorbance (450nm)
0	0.040
15	0.179
30	0.359
60	0.697
120	1.435
240	2.693



#### 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 5 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.
3. 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 level in patients before and after treatment of carcinoma of the pancreas. *J. Clin. Oncol.* 1988; 6:462- 8.
2. Hayakawa, T., Kondo, T., Shibata, T. et al. Sensitive serum markers for detecting pancreatic cancer. *Cancer* 1988; 61:1827-31.
3. Koprowski, H., Herly, M., Steplewski, Z., et al. Specific antigen in serum of patients with colon carcinoma. *Science* 1981; 212:53-5.
4. Malesci, A., Tommasini, M.A., Bonato, C. et al. Determination of CA19-9 antigen in serum and pancreatic juice for differential diagnosis of pancreatic adenocarcinoma from chronic pancreatitis. *Gastroenterology* 1987; 92:60-7.
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 CA 19-9 tumor associated antigen. *American J. of 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; 90:343-9.
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 Res.* 1988; 48:1435-8.
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; 56:2669-73.
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; 1:219-23.
11. Strom BL, Maislin G, West SL, et al. Serum CEA and CA19-9: potential future diagnostic or screening tests for gallbladder cancer? *Int. J. Cancer* 1990; 45:821. 030601