9051 Mira Mesa Blvd, #910094
San Diego, CA 92191
Tel: 760-390-3989

CA 19-9 ELISA Kit

Name	CA 19-9 ELISA Kit
INTENDED USE	The Calbiotech CA19-9 ELISA Kit is intended for the quantitative determination of the Cancer Antigen CA19-9 concentration in human serum or plasma.
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 an adapted solid phase sequential sandwich ELISA. Samples and biotinylated

monoclonal antibody are added to wells coated with streptavidin. CA19-9 in the patient sample binds to biotinylated capture antibody. The biotinylated antibody simultaneously binds to the streptavidin coated plate. After a wash step, anti-CA19-9–HRP enzyme conjugate is added and forms a sandwich around captured CA19-9. Unbound antibodies are washed off. TMB substrate is added resulting in the development of a blue color. The concentration of CA19-9 is directly proportional to the color intensity developed. A standard curve is generated relating color intensity to CA19-9 concentration.

MATERIALS PROVIDED	96 Tests
Microwells coated with streptavidin	12x8x1
Anti CA19-9-Biotin Conjugate, 1 bottle (Ready to use)	12 ml
Anti CA19-9-HRP Enzyme Conjugate, 1 bottle (Ready to use)	12 ml
CA 19-9 Standards, 6 vials (Ready to use)	0.5 ml
TMB Solution, 1 bottle (Ready to use)	12 ml
Stop Solution, 1 bottle (Ready to use)	12 ml
Wash Concentrate 20x, 1 Bottle	25 ml

MATERIALS NOT PROVIDED

Distilled or deionized water

precision pipettes and tips

Disposable pipette tips

Micortiter well reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

Graph paper

STORAGE AND STABILITY

Store the kit at 2 - 8 C.

	Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagents to heat, sun, or strong light.
WARNINGS AND PRECAUTIONS	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 Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. It is recommended that standards and serum samples be run in duplicate. 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. Serum or plasma should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum, plasma-EDTA, or plasma-heparin samples. 2. Specimens may be refrigerated at 2–8C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of samples.
REAGENT PREPARATION	Bring all specimens and kit reagents to room temperature (20-25C) and gently mix. Prepare 1X Wash buffer by adding the contents of the

bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25C) for up to 1 month. Mix well before use.

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25C) and gently mix.

Secure the desired number of coated wells in the holder.

Dispense 25 l of CA19-9 standards, specimens, and controls into appropriate wells.

Dispense 100 µl of anti-CA 19-9-Biotin Reagent (blue color solution) into each well.

Thoroughly mix for 30 seconds at 500-600 rpm. It is very important to mix them completely.

Incubate for 60 minutes at room temperature.

Remove liquid from all wells. Wash each well three times with 350 L of 1X wash buffer. After each wash, sharply and firmly tap the upside down plate on absorbance paper or paper towels to remove residual droplets.

Dispense 100l of anti-CA19-9-HRP Enzyme Conjugate (red solution) into each well.

Incubate for 60 minutes at room temperature.

Remove the contents and wash the plate 3x as described in step 6 above.

Dispense 100 l of the TMB Solution into each well.

Incubate at room temperature for 15 minutes without shaking.

Stop the reaction by adding 50 l of Stop Solution to each well.

Read the absorbance at 450nm (using a reference wavelength of 630nm) with a microtiter plate absorbance reader within 15 minutes.

CALCULATIONS AND RESULTS

Calculate the average absorbance values (A450) 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 via best fit quadratic 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 (U/ml)	Absorbance (450 nm)
0	0.040
25	0.172
75	0.424
150	0.791
300	1.434
600	2.321

LIMITATIONS OF THE PROCEDURE

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.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

REFRENCES

Glenn, J., Steinberg, W.M., Kurtzman, S.H., et at. 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.

Hayakawa, T., Kondo, T., Shibata, T. et al. Sensitive serum markers for detecting pancreatic cancer. Cancer 1988; 61:1827-31.

Koprowski, H., Herly, M., Steplewski, Z., et al. Specific antigen in serum of patients with colon carcinoma.

Science 1981; 212:53-5

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. Gastroenteroglogy 1987; 92:60-

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.

Steinberg, W. The clinical utility of CA 19-9 tumor associated antigen. American J. of Gastroenterology 1990; 85:350-355.

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.

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.

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.

Wang, T.H. Lin, J.W., Chen, D.S., et al. Noninvasive diagnosis of advanced panceatic cancer by real-time ultrasonography, carcinoembryonic antigen, and carbohydrate antigen 19-9. Pancreas 1986; 1:219-23.

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.