

CA19-9 (human) ELISA Kit

(Catalog # K7427-100, 100 assays; Store at 2-8°C)

rev 05/19

I. Introduction:

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 indicate 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. 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. The CA19-9 ELISA test is based on the principle of a solid phase Sandwich enzyme-linked immunosorbent assay. 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-CA-19-9-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.

II. Application:

Quantitative determination of CA19-9

III. Specificity:

Human

IV. Sample Type:

- Serum

V. Kit Contents:

Components	K7427-100	Part No.
Plate coated with streptavidin	12 strips x 8 wells	K7427-100-1
CA19-9 Standard (S1 - S6)	6 x 0.5 ml	K7427-100-2
Anti-CA19-9 Biotin Conjugate	12 ml	K7427-100-3
Anti-CA19-9 HRP Enzyme Conjugate	12 ml	K7427-100-4
Wash Concentrate (20X)	25 ml	K7427-100-5
TMB Substrate	12 ml	K7427-100-6
Stop Solution	12 ml	K7427-100-7

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Concentrate:** Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 25 ml of Wash buffer (20x) in 475 ml of distilled water to prepare 1x Wash buffer. Wash Buffer is stable at room temperature for 1 month (20-25 °C). Mix well before use.

VIII. Warning & Precautions:

- Potential biohazardous materials: The Standard 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, 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.
- 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, control 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.

IX. Sample Preparation and Storage:

Collect blood specimens and separate the serum immediately. Specimens may be stored refrigerated at (2-8° C) for 7 days. If storage time exceeds 7 days, store frozen at (-20° C) for up to six month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic, hemolysis or turbid specimens.

X. Assay Protocol:

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Pipet 25 µl of CA19-9 standards, control and sample into appropriate wells.
3. Add 100 µl of anti-CA 19-9-Biotin Reagent (blue color solution) into each well.
4. Thoroughly mix for 30 seconds at 500-600 rpm. It is very important to mix them completely.
5. Incubate for 60 minutes at room temperature.
6. Remove liquid from all wells. Wash each well three times with 350 µL 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.

7. Add 100µl of anti-CA19-9-HRP Enzyme Conjugate (red solution) into each well.
8. Incubate for 60 minutes at room temperature.
9. Remove the contents and wash the plate 3X as described in step 6 above.
10. Dispense 100 µl of the TMB Solution into each well.
11. Incubate at room temperature for 15 minutes without shaking.
12. Stop the reaction by adding 50 µl of Stop Solution to each well.
13. Read the absorbance at 450 nm (using a reference wavelength of 630 nm) with a microtiter plate absorbance reader within 15 minutes.

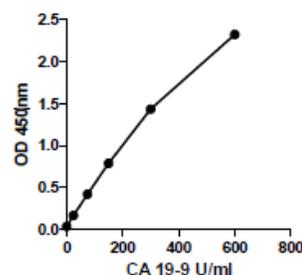
Calculation:

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.

Example of a 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. Standard curve should be run with 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



Expected Values: 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.

XI. RELATED PRODUCTS:

- Carcinoembryonic Antigen (CEA) (human) ELISA Kit (K4805)
- Cancer Antigen 125 (CA-125) (human) ELISA Kit (K4803)
- Cancer Antigen 15-3 (CA15-3) (human) ELISA Kit (K4804)

FOR RESEARCH USE ONLY! Not to be used on humans.