

## Product Information

### CA19-9 ELISA

Catalog Number **SE120154**  
Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

A group of mucin type glycoprotein, Sialosyl Lewis Antigen (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 tissues than in normal or inflamed tissues. Recently, reports indicate 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 serum CA19-9 values may have utility in monitoring subjects with the above-mentioned diagnosed malignancies. It has been shown a persistent elevation in serum CA19-9 value following treatment may be indicative of occult metastatic and/or residual disease. A persistently rising serum CA19-9 value may be associated with progressive malignant disease and poor therapeutic response. A declining CA19-9 value may be indicative of a favorable prognosis and good response to treatment.

The CA19-9 ELISA is an adapted solid phase sequential sandwich ELISA. Samples and biotinylated monoclonal antibody are added to wells coated with streptavidin. CA19-9 in the sample binds to the 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 the 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.

### Components

Materials Provided	96 Tests
Microwells coated with streptavidin	12 x 8 x 1
CA 19-9 Standard set: 6 vials (ready to use)	0.5 mL
CA19-9-Biotin Conjugate Concentrate	12 mL
CA 19-9-HRP Enzyme Conjugate	12 mL
Wash Buffer concentrate (20x)	25 mL
TMB Reagent	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL

### Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes and tips
3. Disposable pipette tips
4. Multiwell plate reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

#### Sample Preparation

1. Serum or plasma should be prepared from a whole blood specimen. This kit is for use with serum, plasma-EDTA, or plasma-heparin samples.
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.

- 1x Wash buffer - Add the contents of the bottle (25 ml, 20x) to 475 ml of distilled or deionized water. Store at room temperature (18–26 °C) for up to 1 month. Mix well before use.

**Storage/Stability**

Store the kit at 2–8 °C.

**Procedure**

Notes: 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 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.

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 25  $\mu\text{L}$  of CA19-9 standards, specimens, and controls into appropriate wells.
3. Dispense 100  $\mu\text{L}$  of CA19-9-Biotin Reagent (blue color solution) into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix them completely.
5. Incubate at room temperature for 60 minutes.
6. Remove the incubation mixture by emptying the plate content into a waste container.
7. Rinse and empty the multiwell plate 3 times with 1x Wash buffer.
8. Strike the multiwell plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100  $\mu\text{L}$  of the CA19-9-HRP Enzyme Conjugate (red-colored solution) into each well. Mix gently for 30 seconds.
10. Incubate at room temperature for 60 minutes.
11. Remove the incubation mixture by emptying the plate content into a waste container.
12. Rinse and empty the multiwell plate 3 times with 1x Wash buffer.
13. Strike the multiwell plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
14. Dispense 100  $\mu\text{L}$  of the TMB Reagent into each well. Gently mix for 10 seconds.
15. Incubate at room temperature in the dark for 15 minutes without shaking.
16. Stop the reaction by adding 50  $\mu\text{L}$  of Stop Solution to each well.
17. Read the optical density at 450 nm with a multiwell plate reader within 15 minutes.

## Results

### Calculations

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 a standard curve

Typical results of a typical standard run with optical density readings at 450 nm. These results are 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

**Note:** Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the 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. 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

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