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Catalog No. CA238T (96 Tests)

# **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. For Research Use Only. For professional use only. Not for use in diagnostic procedures.

## SUMMARY AND EXPLANATION

CA19-9 is a sialylated Lewis antigen found on glycolipids and glycoproteins. In research, it is used for studies in glycobiology, antigen—antibody binding, carbohydrate epitope mapping, and as part of glycan-related biomarker research panels.

## 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
1.	Microwells coated with streptavidin	12x8x1
1.	Anti CA19-9-Biotin Conjugate, 1 bottle (Ready to use)	12 mL
2.	Anti CA19-9-HRP Enzyme Conjugate, 1 bottle (Ready to use)	12 mL
3.	CA 19-9 Standards, 6 vials (Ready to use)	0.5 mL
4.	CA 19-9 Controls, 2 vials (Ready to use)	0.5 mL
5.	TMB Solution, 1 bottle (Ready to use)	12 mL
6.	Stop Solution, 1 bottle (Ready to use)	12 mL
7.	Wash Concentrate 20x, 1 Bottle	25 mL

## MATERIALS NOT PROVIDED

- Distilled or deionized water
- 2. precision pipettes and tips
- 3. Disposable pipette tips
- 4. Microtiter well reader capable of reading absorbance at 450nm
- 5. Absorbance paper or paper towel
- Graph paper

# STORAGE AND STABILITY

- 1. Store the kit at 2 8° C.
- 2. Keep microwells sealed in a dry bag with desiccants.
- 3. The reagents are stable until expiration of the kit.
- 4. Do not expose reagents to heat, sun, or strong light.



#### WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

- The standards 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.
- 3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- 4. 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.

# SPECIMEN COLLECTION AND HANDLING

This assay is designed for use with human serum or plasma samples obtained in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Samples may be stored refrigerated (2–8 °C) for up to seven days, or frozen (–20 °C or below) for up to six months. Avoid repetitive freeze—thaw cycles.

#### REAGENT PREPARATION

Bring all specimens and kit reagents to room temperature (20-25 °C) 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 (20-25 °C) for up to 1 month. Mix well before use.

#### ASSAY PROCEDURE

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

- 1. Secure the desired number of coated wells in the holder.
- 2. Dispense 25 µl of CA19-9 standards, specimens, and controls into appropriate wells.
- 3. Dispense 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.
- 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. Dispense 100ul 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  $\mu$ I of Stop Solution to each well.
- 13. 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.
- 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.

IFU-CA238T-RC-V2

## **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.