

## REFERENCES

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## CA19-9 ELISA

Catalog No. CA238T (96 Tests)

### INTENDED USE

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

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.	TMB Solution, 1 bottle (Ready to use)	12 ml
5.	Stop Solution, 1 bottle (Ready to use)	12 ml
6.	Wash Concentrate 20x, 1 Bottle	25 ml

### MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. precision pipettes and tips
3. Disposable pipette tips
4. Micortiter well reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. 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

1. For Research Use Only. Not for use in diagnostic procedures.
2. For Laboratory use.
3. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different kit lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care, since it is corrosive.
15. Bring all reagents to room temperature.

Cat#: CA238T (96 Tests)

For Order and Inquiries, please contact



Calbiotech Inc.,

1935 Cordell Ct., El Cajon, CA 92020

Tel (619) 660-6162, Fax (619) 660-6970,

[www.calbiotech.com](http://www.calbiotech.com)

- Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
- Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

### SPECIMEN COLLECTION AND HANDLING

- 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.
- Typically, 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 samples.

### 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.

- Secure the desired number of coated wells in the holder.
- Dispense 25 µl 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 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.
- Dispense 100µl 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 µl of the TMB Solution into each well.
- Incubate at room temperature for 15 minutes without shaking.
- Stop the reaction by adding 50 µl 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.

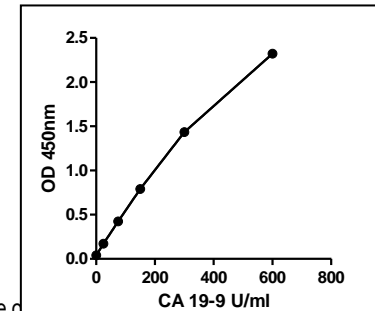
### CALCULATIONS AND RESULTS

- 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 only when used in accordance with the instructions provided 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.