ab108642

Cancer Antigen CA19-9 Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human cancer antigen CA19-9 concentrations in serum

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Introduction

ab108642 Cancer Antigen CA19-9 Human ELISA Kit is intended for the quantitative determination of the Cancer Antigen CA19-9 concentration in Human serum.

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. Cancer Antigen CA19-9 represents the most important and basic carbohydrate tumor marker. The immunohistologic distribution of Cancer Antigen CA19-9 in tissues is consistent with the quantitative determination of higher CA19-9 concentrations in cancer than in normal or inflamed tissues. Recent 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.

Research studies demonstrate that serum CA19-9 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 Cancer Antigen CA19-9 value may be associated with progressive malignant disease and poor therapeutic response. A declining Cancer Antigen CA19-9 value may be indicative of a favorable prognosis and good response to treatment.

2. Assay Summary

ab108642 is based on the principle of a solid phase enzyme-linked immunosorbent assay.

The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact Cancer Antigen CA19-9 molecule for solid phase immobilization (on the microtiter wells). Another Cancer Antigen CA19-9 monoclonal antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution.

The test sample is allowed to react sequentially with the two antibodies, resulting in the Cancer Antigen CA19-9 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After two separate incubation steps at 37°C for 90 minutes, the wells are washed with Wash Buffer to remove unbound labeled antibodies.

A solution of tetramethylbenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of a blue color.

The color development is stopped with the addition of 1N hydrochloric acid (HCl) changing the color to yellow. The concentration of Cancer Antigen CA19-9 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

3. Kit Contents

- Antibody-Coated Wells (1 plate, 96 wells); microtiter wells coated with Murine monoclonal anti-Cancer Antigen CA19-9.
- Cancer Antigen CA19-9 reference standards (1-6) containing 0, 25, 75, 150, 300, and 600 U/ml CA19-9, liquid, 0.5 ml each, ready to use. 1 set.
- Cancer Antigen CA19-9 Assay Buffer, 13 ml.
- Enzyme Conjugate Concentrate (12X), 1.1 ml.
- Cancer Antigen CA19-9 Conjugate Diluent, 13 ml.
- Wash Buffer Concentrate (20X), 50 ml.
- TMB Reagent (11 ml) contains one-step TMB solution.
- Stop Solution (11 ml) contains diluted hydrochloric acid (1N HCl).

4. Storage and Handling

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above.

5. Additional Materials Required

- Distilled or deionized water
- Precision pipettes: 100 µl and 200 µl.
- Disposable pipette tips
- Microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at a wavelength of 450 nm.
- Vortex mixer, or equivalent
- Absorbent paper or paper towel
- Graph paper

6. Preparation of Reagents

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. To prepare **Wash Buffer (1X)**: Add 50 ml of Wash Buffer (20X) to 950 ml of DI water. The diluted Wash Buffer is stable at 2-8°C for 30 days. Mix well before use. Note: Any crystals that may be present due to high salt concentration must be redissolved at room temperature before making the dilution.
3. To prepare **Working Cancer Antigen CA19-9 Conjugate Reagent:**

- For 3.0 ml, which is more than enough for 24 wells: Add 0.25 ml of Conjugate Concentrate (12x) to 2.75 ml of the Enzyme Conjugate Diluent (1:11 dilution) and mix well.
- For 6.0 ml, which is more than enough for 48 wells: Add 0.5 ml of Conjugate Concentrate (12x) to 5.5 ml of the Enzyme Conjugate Diluent (1:11 dilution) and mix well.
- For 9.0 ml, which is more than enough for 72 wells: Add 0.75 ml of Conjugate Concentrate (12x) to 8.25 ml of the Enzyme Conjugate Diluent (1:11 dilution) and mix well.
- For 12.0 ml, which is more than enough for 96 wells: Add 1.0 ml of Conjugate Concentrate (12x) to 11.0 ml of the Enzyme Conjugate Diluent (1:11 dilution) and mix well.

The Working* Cancer Antigen *CA19-9 Conjugate Reagent needs to be prepared freshly every time before use. The amount of conjugate diluted depends on your assay size. Discard the excess after use.

7. Preparation and Collection of Specimen

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only

8. Assay Method

1. Secure the desired number of coated wells in the holder.
2. Dispense 10 µl of Cancer Antigen CA19-9 standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Cancer Antigen CA19-9 Assay Buffer (green-color solution) into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix them completely.
5. Incubate at 37°C for 90 minutes.
6. Remove the incubation mixture by emptying the plate content into a waste container.
7. Rinse and flick the microtiter wells 5 times with **Wash Buffe**r **(1X)**.
8. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of the **Working Conjugate Reagent** (red-colored solution) into each well. Mix gently for 30 seconds.
10. Incubate at 37°C for 90 minutes.
11. Remove the incubation mixture by emptying the plate content into a waste container.
12. Rinse and flick the microtiter wells 5 times with **Wash Buffer (1X).**
13. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.

14. Dispense 100 µl of the TMB Reagent into each well. Gently mix for 10 seconds.
15. Incubate at room temperature in the dark for 20 minutes without shaking.
16. Stop the reaction by adding 100 µl of Stop Solution to each well.
17. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
18. Read the optical density at 450 nm with a microtiter plate reader ***within 15 minutes.***

9. Data Analysis

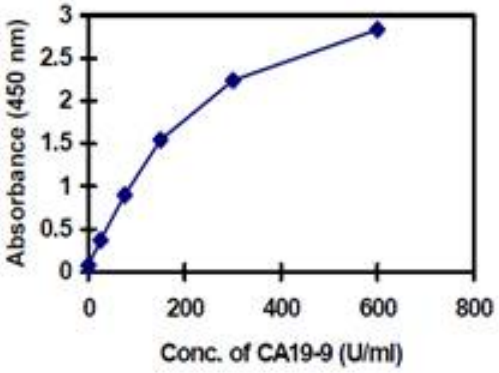
1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml via best fit quadratic on linear graph paper, with absorbance on 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 Cancer Antigen CA19-9 in U/ml from the standard curve.

A. Typical Data

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against Cancer Antigen CA19-9 concentrations shown in the X axis.

NOTE: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

CA19-9 (U/ml)	Absorbance (450 nm)
0	0.075
25	0.373
75	0.900
150	1.543
300	2.237
600	2.832



B. Sensitivity

The minimum detectable concentration of Cancer Antigen CA19-9 in this assay is estimated to be 10 U/ml.

C. Expected Values

Healthy men and women are expected to have Cancer Antigen CA19-9 assay values below 35 U/ml.

10. Limitations

- 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.
- 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.

11. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4 °C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution

Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

UK, EU and ROW
Email:
technical@abcam.com
Tel: +44 (0)1223 696000
www.abcam.com

US, Canada and Latin America
Email: us.technical@abcam.com
Tel: 888-77-ABCAM (22226)
www.abcam.com

China and Asia Pacific
Email: hk.technical@abcam.com
Tel: 400 921 0189 / +86 21 2070 0500
www.abcam.cn

Japan
Email: technical@abcam.co.jp
Tel: +81-(0)3-6231-0940
www.abcam.co.jp