CA 19-9 ELISA Kit

Cat. No. 99-0070

Size: 96 Tests

For Research Use Only

Lot No.

The CA 19-9 ELISA Kit is an enzyme-linked immunosorbent sandwich assay for quantitative detection of human CA 19-9 in human serum.

INTRODUCTION

Cancer antigen 19-9 (CA 19-9 3) is the most important and basic carbohydrate tumor marker. CA19-9 is a sensitive marker for pancreatic, gastric and hepatobiliary malignancies. The serum CA 19-9 level is frequently elevated in the serum of subjects with various gastrointestinal malignancies, such as pancreatic, colorectal, gastric and hepatic carcinomas.

REAGENTS AND MATERIALS PROVIDED

96-well microwell plate coated with monoclonal antibody against CA 19-9
Assay Buffer: 13 mL
Standard: 6 vials containing 0, 25, 75, 150, 300, 600 Unit/mL, Ready to use
Enzyme Conjugate (12X): 2 mL
Enzyme Conjugate Diluent: 24 mL
TMB Substrate: 1 vial (11 mL), Ready to use
Stop Solution: 1 vial (11 mL), Ready to use

MATERIALS REQUIRED BUT NOT PROVIDED

5 mL and 10 mL graduated pipettes, beakers, flasks, and cylinders
10 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
50 µL to 300 µL adjustable multichannel micropipette, disposable tips, and reservoir
Microwell strip reader capable of reading at 450 nm

STORAGE

2° - 8°C

PRINCIPLE OF TEST

This ELISA kit is based on a solid phase sandwich ELISA method. Samples and diluent are added to the wells coated with monoclonal antibody against CA 19-9. CA 19-9 in the serum binds to antibody coated on the well. Unbound proteins are washed off. HRP-labeled anti-CA 19-9 antibody is then added to the mixture. Unbound conjugates are washed off. Upon the addition of the substrate, the intensity of color is proportional to the concentration of CA 19-9 in the samples. A standard curve is prepared by plotting color intensity and the concentration of the CA 19-9.

PREPARATION OF REAGENTS

All reagents should be brought to room temperature before use.
CA 19-9 conjugate reagent: Add the entire 2.0 mL of conjugate concentrate (12x) to 22 mL of the Enzyme Conjugate Diluent (1:11 dilution) and mix well. The working CA 19-9 Conjugate Reagent must be prepared freshly each time before use. Discard excess after use.

PROCEDURE

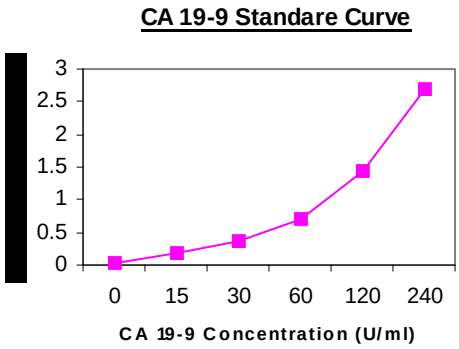
Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Pipet 100µL of CA 19-9 standards, controls and samples in appropriate wells.
2. Pipet 100µL of Assay buffer into each well. Mix gently for 30 seconds.
3. Cover the plate and incubate for 1.5 hours at 37˚C.
4. Remove the incubation mixture from all wells. Wash wells five times with distilled water. Blot on absorbent paper towels.
5. Pipet 200µL of Enzyme Conjugate into each well. Mix well.
6. Cover the plate and incubate for 1.5 hours at 37˚C.
7. Remove liquid from all wells. Wash wells with distilled water for five times. Blot on absorbent paper towels.
8. Add 100µL of TMB Substrate into each well. Gently mix for 10 second.
9. Incubate for 20 minutes at room temperature in the dark without shaking.
10. Add 100µL of Stop Solution into each well.
11. Gently mix for 30 seconds. Make sure that the blue color completely changes to yellow.
12. Read absorbance on ELISA Reader at 450 nm within **15 minutes** after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Calculate the average absorbance values (A450) for each set of reference standards, control and samples.
2. To construct the standard curve, plot the absorbance for the CA 19-9 standards (vertical axis) against its concentration in ng/mL (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Use the absorbance for controls and each unknown sample to determine the corresponding concentration of CA 19-9 from the standard curve.



SENSITIVITY

The sensitivity of this kit is estimated to be 5 U/mL.