

CA19-9 ENZYME IMMUNOASSAY TEST KIT

Catalog Number: EA100980



Enzyme Immunoassay for the Quantitative Determination of CA 19-9 in Serum

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

PRINCIPLE OF THE TEST

The CA19-9 ELISA test 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 CA19-9 molecule is used for solid phase immobilization (on the microtiter wells). Another CA 19-9 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 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 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 Stop Solution changing the color to yellow. The concentration of CA19-9 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the test kits:

- Murine monoclonal anti-CA19-9 coated 96 well microtiter plate.
- CA19-9 reference standards, containing 0, 25, 75, 150, 300, and 600 U/ml CA19-9, liquid, 0.5 ml each, ready to use. 1 set.
- CA 19-9 Assay Buffer, 13 ml
- Enzyme Conjugate Concentrate (12X), 1.1 ml
- CA 19-9 Conjugate Diluent, 13 ml
- Wash Buffer Concentrate (20X), 50 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

STORAGE OF TEST KIT AND

INSTRUMENTATION

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. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be brought to 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 CA 19-9 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 CA 19-9 Conjugate Reagent needs to be prepared freshly every time before use.

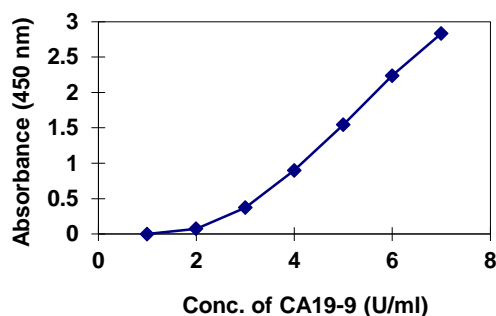
The amount of conjugate diluted depends on your assay size. Discard the excess after use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense **10 μ l** of CA19-9 standards, specimens, and controls into appropriate wells.
3. Dispense **100 μ l** of CA 19-9 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 Buffer (1X)**.
8. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense **100 μ l** of the **Working Conjugate Reagent** 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 μ l 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 μ l 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.***

300	2.237
600	2.832



TECHNICAL CONSULTATION

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CALCULATION OF RESULTS

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.

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EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm 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	0.075
25	0.373
75	0.900
150	1.543

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