Carbohydrate antigen 19-9(CA199) ELISA Test Kit

NAME AND INTENDED USE

Detection Kit for Carbohydrate antigen 19-9(CA199)(ELISA). It is used in quantitative tests for CA19-9 in human serum.

SUMMARY AND CLINICAL SIGNIFICANCE

Carbohydrate antigen 19-9 (CA19-9) is a tumor-associated antigen oligosaccharide and also called pancreatic cancer antigen or sialylated Lewis antigen. CA19-9 is a molecular weight over 5000, 000 and its epitopes is formed of glycoproteins with mucin in serum. CA19-9 present in colorectal cancer cells, and also present in the cancer tissue of pancreas, lung, thyroid, ovary, prostate.

This reagent mainly is used for dynamic monitoring of cancer patients to determine disease progression or auxiliary therapeutic effect, but its concentration is not correlated with tumor size, growth, and the degree of malignancy grading/staging. In some benign diseases (Such as acute pancreatitis, cholecystitis, cholangitis cholestasis, hepatitis, etc.) can also be seen that the index increased in varying degrees. Therefore, the CA19-9 should not be considered an effective general screening test for the general population of cancer screening, early diagnosis and other purposes.

PRINCIPLE

CA-19-9 uses a “sandwich principle”, Enzyme-linked immunological sorbent assay. To measure CA-19-9 levels in serum, plastic wells coated with a monoclonal antibodies of CA-19-9 are supplied in the kit. After the patient’s specimen and another mono-antibody labelled with HRP are added, CA-19-9, if present, is fixed to the solid phase antibody and creating a HRP-antibody—CA-19-9—antibody “sandwich”. After TMB substrate added, the result is obtained by EIA plate reader.

PRECAUTION FOR USERS

1. Handling should preclude any pipetting by mouth.
2. Use only pipettes with disposable tips for each specimen.
3. Do not mix materials from different master lots. Do not use kit components beyond the expiration date. All materials should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

Serum specimens can be tested by the CA-19-9 procedure. Remove serum from the clot as soon as possible to avoid hemolysis. Covered specimens can be stored up to 48 hours at 2~8°C. Specimens held for a longer time can be frozen at –20°C and avoid repeated freeze melting.

Serum samples with concentrations expected to be greater than 150 U/ml should be diluted with normal saline.

NOTE: If needed, remove by centrifugation the suspended fibrin particles or aggregates which are liable to produce falsely positive results.

REAGENTS SUPPLIED

1. Coated Microplate: 1 plate (8×12 wells), Ready to use. Coated with anti-CA19-9 antibody and sealed in an aluminum bag. Remove the strips in the resealable bag with a desiccant to protect from moisture after opened. Store at 2~8°C until expiration date.
2. HRP Conjugate: 1 vial of 6ml, Ready to use. Store at 2~8°C until expiration date.
3. Calibrator: 5 vials of 0.5ml, Ready to use. Labeled with S0 to S4 and the concentration of CA19-9 is 0, 10, 40, 100, 150 U/ml. Store at 2~8°C until expiration date.
4. Control: 1 vial of 0.5ml, Ready to use. Store at 2~8°C until expiration date. The concentration is 39.4~73.2 U/ml.
5. Chromogen A: 1 vial of 7ml, Ready to use. Store at 2~8°C until expiration date.
6. Chromogen B: 1 vial of 7ml, Ready to use. Store at 2~8°C until expiration date.
7. Stop Solution: 1 vial of 7ml, Ready to use. Store at 2~8°C until expiration date.
8. Wash buffer: 1 vial of 15ml, Concentrate 20-fold, diluting with deionized water before the assay. Store at 2~8°C until expiration date.
9. Plate sealer: 2 pieces.
10. Plastic resealable bag: 1 set.
11. Instruction manual: 1 copy.

RELATED TIPS

1. This kit is designated for In-Vitro Diagnostic Use Only.
2. Wash procedure. Incomplete washing will adversely affect the test results. Wash each well 3 times with about 0.3ml wash buffer. If no automatic washer is available, washing can be performed manually as follows: Invert the plate vigorously to get all wash buffer out and block the rim of well on absorbent paper for a few seconds. Filling each well with wash buffer and remain 10 seconds. Repeat these steps 3 times. Blot dry the plate by inverting the plate onto absorbent tissue, and striking a hard surface several times.
3. Drip procedure. Mix the bottle gently before use. Violent surge may cause too much foam. Invert the bottle and squeeze one or two drop on absorbent tissue to make sure there is no foam. Take the bottle upright the well and make sure the drop does not touch the rim of wells.
4. Read procedure. Using the blank well to correct the zero point of reader if single wavelength reader is used. If double wavelength readers with 450nm and 630nm are used, there is no need to correct the zero point.
5. Storage. The whole kit should be stored at 2~8°C for one year. Microplate should be taken to room temperature before opened. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. After removingthe required number of strips, the plate should be put in the plastic resealable bag with desiccants to minimize exposure to damp air.
6. Control serum is prepared with human serum, which is tested negative of HBV, HCV and HIV. But it should still be considered as capable of transmitting viral diseases.

PREPARATIONS

1. Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use.
2. Prepare Wash Buffer by diluting Wash Concentrate 20-fold with deionized water. The diluted wash solution is stable in room temperature for at least one week.

ASSAY PROCEDURE:

1. Mark the microtitration strips to be used. All the calibrators and controls should set duplicate.
2. Dispense 50 ul of calibrators/controls/samples into wells.
3. Dispense 50 ul of HRP Conjugate to each well.
4. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 1 hour.
5. Wash each well for 3 times, 10 seconds each time. (See wash procedure).
6. Dispense 50 ul of chromogen A to each well.
7. Dispense 50 ul of chromogen B to each well.
8. Covered the strips with a fresh plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 15 minutes.
9. Dispense 50 ul of stopping solution to each well and mix completely.
10. Read the absorbance of the plate within 10 minutes. (See read procedure)

CALCULATION OF RESULTS

Computer: Use the linear fitting function, the logarithm of each calibrator concentration (Log), as X, take the logarithm of the corresponding absorbance value (Log(OD)) as Y, choose double logarithm (or full Logarithmic) Log-Log fitting the concentration of the serum to be tested is calculated from the fitted line.

Equation: log OD=B*log [concentration]+A

PERFORMANCE CHARACTERISTICS

1. Expected value

The cut-off value of CA19-9 concentration for healthy subjects is 37U/ml.

2. Sensitivity

The detection limit of the assay is approximately 3.8 U/ml.

3. Precision

Interassay≤20%

Intraassay≤15%

4. Accuracy

The recovery rate is 85%～115%.

5. High-Dose-Hook Effect

No hook effect was observed in this test up to 8000U/ml.

CODIGO: RSET114-2