



Human CA19-9 ELISA Kit

Enzyme Immunoassay for the quantification of human CA19-9 in serum and plasma.

Catalog number: ARG80645

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

The sialyl Lewis_x epitope is also known as the cancer associated antigen CA 19-9. CA 19-9 levels are frequently elevated in the serum of patients with cancers of the pancreato-biliary system (i.e. pancreas, gallbladder, biliary tract). In addition, elevated levels of CA 19-9 have been observed in other malignancies such as lung cancer, other gastrointestinal cancers and in some nonmalignant disorders. It must be taken into account that patients, genotypically negative for the Lewis blood group antigen, will be unable to produce the CA 19-9 antigen even in the presence of malignant tissue.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CA 19-9 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any CA 19-9 present is bound by the immobilized antibody. After washing away any unbound substances, a Horseradish Peroxidase (HRP)-conjugated antibody specific for CA 19-9 is added to each well and incubated. Following a washing to remove unbound substances, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of CA 19-9 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm \pm 2 nm. The concentration of CA 19-9 in the sample is then determined by comparing the O.D of samples to the standard curve.

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MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate	4°C
Zero standard (S0)	3 ml (ready to use)	4°C
Standard 1-5 (15, 30, 60, 120, 240 U/ml)	5 x 0.5 ml (ready to use)	4°C
Control low	0.5 ml (lyophilized)	4°C, lyophilized
Control high	0.5 ml (lyophilized)	4°C, lyophilized
HRP conjugated antibody	14 ml (ready to use)	4°C
Assay Buffer	7 ml (ready to use)	
40X Wash buffer	30 ml	4°C
TMB substrate	14 ml (ready to use)	4°C (Protect from light)
STOP solution	14 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Store the unopened reagents at 2- 8°C until expiration date. Once opened the reagents are stable for 2 month when stored at 2 – 8°C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.
- All kit reagents and specimens should be brought to room temperature (21-26°C) and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- If crystals are observed in the 40X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Briefly vortex or mix and spin down the components before use.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn

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solution colored. Do not pour reagents back into vials as reagent contamination may occur.

- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Storage: Specimens may be stored for up to 5 days at 2°C to 8°C and up to 18 months while stored at -20°C . Samples should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Note: Do not use haemolytic, icteric or lipaemic specimens.

Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash buffer:** Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. The diluted Wash Solution is stable for 2 weeks at room temperature.
- **Samples:** If the initial assay found samples contain CA19-9 higher than the highest standard, the samples can be diluted with Standard 0 (S0) and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

- a) Dilution 1:10: 10 μ L Serum + 90 μ L Standard 0 (mix thoroughly).
- b) Dilution 1:100: 10 μ L 1:10 diluted a) + 90 μ L Standard 0 (mix thoroughly).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 21-26°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50 μ L of standards, controls and samples in duplicate into sample wells.
3. Add 50 μ L Assay Buffer into each well. Incubate for 60 minutes at RT.
4. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1 \times Wash Buffer (350 μ L) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of

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liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Add 100 μ l of HRP conjugated antibody into each well. Incubate for 60 minutes at RT.
6. Wash as according to step 4.
7. Add 100 μ l of TMB mixture to each well. Incubate for 30 minutes at room temperature in dark.
8. Add 100 μ l of Stop Solution to each well.
9. Read the OD with a microplate reader at 450 nm immediately. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value 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 from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give

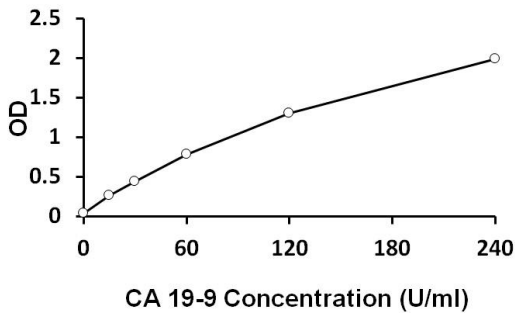
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slightly different results.

5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 240 U/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The analytical sensitivity of CA 19-9 ELISA kit was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Zero Standard and was found to be 0.2 U/mL

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 9.07% and inter-assay precision was 6.23%.

Recovery

85.5-114.7%

Interfering Substances

Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 30 mg/ml) have no influence on the assay results.