Human CA19-9 ELISA Kit

For the quantitative determination of human Carbohydrate Antigen 19-9 (CA19-9) concentrations in serum, plasma and tissue supernatant

Catalogue Number: EL10061

96 Wells

FOR LABORATORY RESEARCH USE ONLY
NOT FOR DIAGNOSTIC USE

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S7.5 (0)

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INTENDED USE

This Human CA19-9 ELISA Kit is to be used for the *in vitro* quantitative determination of human CA19-9 concentrations in serum, plasma, and tissue supernatant. This kit is intended FOR LABORTORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Carbohydrate antigen 19-9 (CA19-9), a tetrasaccharide also known as sialyl Lewis^A, is abundantly expressed on the surface of pancreatic cancer cells, as well as on the surface of colorectal and other cancer cells. Serum CA19-9 usually ranges from 0 to 37 units per milliliter in healthy population. Higher than normal level is often an indication of cancer in the pancreas, gallbladder, live, lung, or colon. Serum CA19-9 is an extensively validated, and currently, the most important biomarker available for monitoring pancreatic cancer progression and regression. CA19-9 serum levels have been applied clinically for predicating chemotherapy response, post operational recurrence, survival rate statistics and other prognostic information. It is worthy of note that pancreatic cancers with a Lewis-negative phenotype (5-10%) can produce false-negative results. This CA19-9 ELISA kit uses a pair of monoclonal antibodies that was developed by immunization with CA19-9 expression cancer cell line SW1116 and screened with highly purified reference-grade native CA19-9, thus warranting assay specificity. The performance data of this kit demonstrated high accuracy and sensitivity, and low coefficient of variation.

This CA19-9 ELISA is a 2.5-hour solid phase enzyme linked immunoassay readily applicable to measure CA19-9 in serum, plasma, and cell culture supernatant. This CA19-9 ELISA is expected to be effectively used for further investigations into the relationship between CA19-9 and the various conditions mentioned above.

PRINCIPLE OF THE ASSAY

This CA19-9 enzyme-linked immunosorbent assay (ELISA) applies a technique called quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for CA19-9. Standards or samples are then added to the appropriate microtiter plate wells and incubated. CA19-9, if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove any unbound CA19-9 and other components of sample. In order to quantitate the amount of CA19-9 present in the sample, a horseradish peroxidase(HRP)-conjugated monoclonal antibody specific for CA19-9 is added to each well to "sandwich" the CA19-9 immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate solution are allowed to react over a short incubation period. The wells that contain CA19-9 will exhibit blue colour. The enzyme-substrate reaction is terminated by the addition of

sulphuric acid solution and the colour will change to yellow. The optical density will be measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of CA19-9 in the samples, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time. This allows the operator to produce a standard curve of Optical Density (O.D) versus CA19-9 unit/mL (U/mL). The concentration of CA19-9 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

	96 tests

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

1. **CA19-9 MICROTITER PLATE** (Part EL61-1) ***96 wells***
   Pre-coated with anti-human CA19-9 monoclonal antibody

2. **CA19-9 CONJUGATE** (Part EL61-2) ***15 mL***
   Anti-human CA19-9 monoclonal antibody conjugated to horseradish peroxidase with preservative

3. **CA19-9 STANDARD** (Part EL61-3) ***2 vials***
   Human CA19-9 (400 units per vial) in a buffered protein base with preservative, lyophilized.

4. **CALIBRATOR DILUENT** (Part EL61-4) ***25 mL***
   Animal serum with preservative, for standard and sample dilution

5. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

6. **SUBSTRATE A** (Part EL61-5) ***11 mL***
   Buffered solution with H_2O_2.

7. **SUBSTRATE B** (Part 30007) ***11 mL***
   Buffered solution with TMB.

8. **STOP SOLUTION** (Part 30008) ***14 mL***
   2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100µL or 50-200µL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the virus before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone. Keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form, which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING AND STORAGE OF SERUM AND PLASMA

a) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.

b) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, heparin or CDPA as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4°C) to remove any particulate.

- Avoid hemolytic, lipidic or turbid samples.
- Serum and plasma samples to be used within 24-48 hours may be stored at 2-8°C. Otherwise, samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

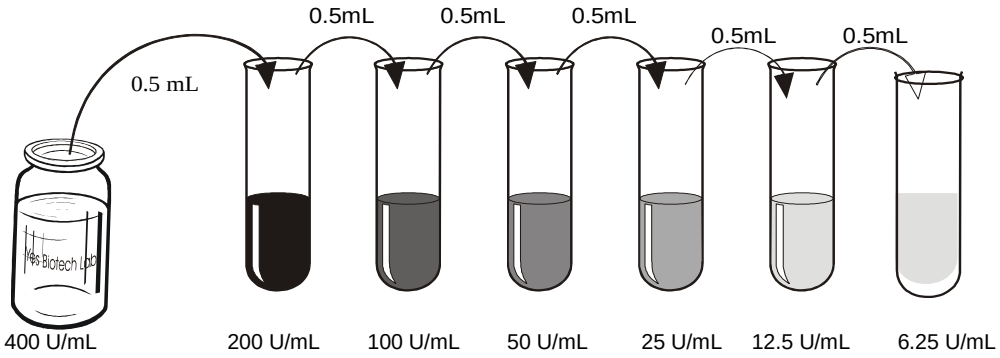
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **CA19-9 Standard:**

a) Reconstitute CA19-9 Standard with 1.0mL of Calibrator Diluent for serum/plasma testing. This reconstitution produces a stock solution of 400 U/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The CA19-9 standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles. Aliquot if repeated use is expected.

b) Use the above stock solution to produce a 2-fold dilution series within the range of this assay (6.25 U/mL to 400 U/mL) as illustrated below. Add 0.5mL Calibrator Diluent to each test tube. Between each test tube transfer, be sure to mix contents thoroughly. The undiluted CA19-9 Standard will serve as the high standard (400 U/mL) and the Calibrator Diluent will serve as the zero standard (0 U/mL).



ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and CA19-9 Standards before starting assay procedure (see Preparation of Reagents). *The table and diagram provided below can be used as a reference for adding Standards and Samples to the Microtiter Plate.*

Wells	Contents	**Wells**	Contents
1A, 1B	Standard 1 - **0 U/mL** (S1)	**2A, 2B**	Standard 5 - **50 U/mL** (S5)
1C, 1D	Standard 2 – **6.25 U/mL** (S2)	**2C, 2D**	Standard 6 - **100 U/mL** (S6)
1E, 1F	Standard 3 – **12.5 U/mL** (S3)	**2E, 2F**	Standard 7 - **200 U/mL** (S7)
1G, 1H	Standard 4 – **25 U/mL** (S4)	**2G, 2H**	Standard 8 – **400 U/mL** (S8)
3A, 12H	Samples		

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	1	5	9	13	17	21	25	29	33	37
B	S1	S5	1	5	9	13	17	21	25	29	33	37
C	S2	S6	2	6	10	14	18	22	26	30	34	38
D	S2	S6	2	6	10	14	18	22	26	30	34	38
E	S3	S7	3	7	11	15	19	23	27	31	35	39
F	S3	S7	3	7	11	15	19	23	27	31	35	39
G	S4	S8	4	8	12	16	20	24	28	32	36	40
H	S4	S8	4	8	12	16	20	24	28	32	36	40

2. Add 50µL of calibrator diluent to each well of anti-human CA19-9 antibody pre-coated microtiter plate. Add 50µL of Standards or Samples to the appropriate wells. Mix briefly on ELISA plate shaker. Cover and incubate for **1 hour at room temperature**.

3. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

4. Add 100µL of conjugate to each well. Cover and incubate for **1 hour at room temperature**.

5. Prepare Substrate Solution (see Preparation of Reagents) **no more than 15 minutes** before end of second incubation.

6. Repeat wash procedure as described in Step 3.

7. Add 100μL Substrate Solution to each well. Cover and incubate for **15 minutes at room temperature**.

8. Add 100μL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader **within 10 minutes.**

CALCULATION OF RESULTS

The standard curve is used to determine the amount of CA19-9 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding CA19-9 concentration (U/mL) on the horizontal (X) axis.

1. Construct the standard curve using graph paper or statistical software.

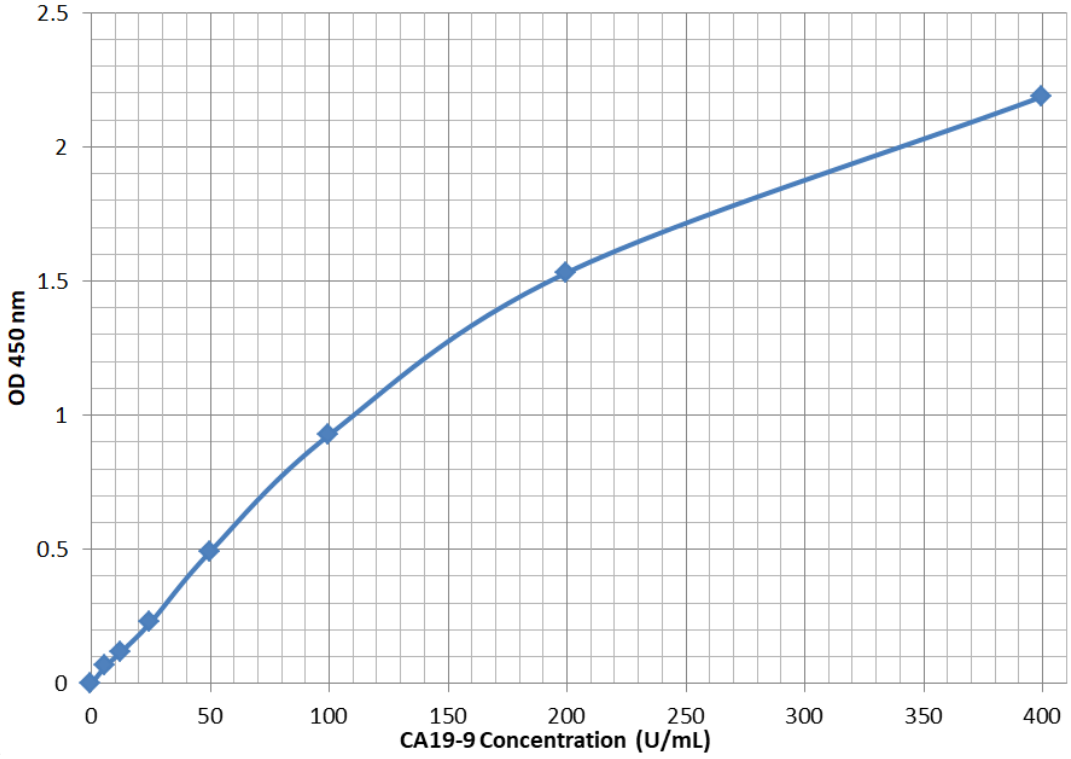
2. To manually determine the amount of CA19-9 in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding CA19-9 concentration in sample.

3. If statistic software (program) is available, it is recommended to select “Point to Point” or “Four Parameter Logic” method to obtain the standard curve for accurate sample value determination.

4. If a tested sample generates value higher than or close to the highest standard, make a 10 fold further dilution from the diluted samples with CA19-9 Calibrator Diluent, and repeat the assay. The measured value is multiplied with dilution factor to obtain the final concentration for the sample.

TYPICAL DATA

Standard (U/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.052, 0.050	0.051	0
6.25	0.121, 0.116	0.119	0.068
12.5	0.175, 0.162	0.169	0.118
25	0.288, 0.274	0.281	0.230
50	0.535, 0.553	0.544	0.493
100	0.965, 0.990	0.978	0.927
200	1.574, 1.592	1.583	1.532
400	2.231, 2.247	2.239	2.188



PERFORMANCE CHARACTERISTICS

1. Intra-assay precision

To determine within-run precision, two diluted samples of known concentration were assayed by measuring 8 replicates in 1 assay.

Sample	**1**	**2**
N	8	8
Mean (U/mL)	15.567	83.479
Standard Deviation (OD)	0.009	0.041
Coefficient of Variation (%)	*2.532*	3.512

2. Inter-assay precision

To determine between-run precision, two diluted samples of known concentration were measured in 8 different assays.

Sample	**1**	**2**
N	8	8
Mean (U/mL)	12.65	84.60
Standard Deviation (U/mL)	0.77	3.60
Coefficient of Variation (%)	6.10	4.18

3. Intra-assay Accuracy

Intra-assay accuracy was validated by 8 repeated measurements of serial diluted samples at 5 concentration levels covering the calibration range in one assay. The intra-assay accuracy of the test was estimated by comparing the average value of the measured samples with nominal value.

Sample Dilution	Nominal value (U/ml)	Four Parameter Logic curve fitting		
		Measured values (U/ml)	CV (%)	Percentage of nominal value (%)
1:100	142.98	148.425	4.7	97.3
1:200	71.49	65.494	2.5	91.6
1:400	35.75	33.179	3.2	92.8
1:800	17.87	16.946	3.1	94.8
1:1600	8.935	8.306	4.2	92.9

4. Inter-assay Accuracy

Inter-assay accuracy was validated by analyzing serial diluted samples at 5 concentration levels in 8 runs of three days. The inter-assay accuracy of the test was estimated by comparing the average value of the measured samples with nominal value.

Sample Dilution	Nominal value (U/ml)	Four Parameter Logic curve fitting		
		Range of measured values (U/ml)	CV (%)	Percentage (average) of nominal value (%)
1:100	142.98	109.27-148.42	7.6	90.9

1:200	71.49	60.785-74..07	5.7	97.6
1:400	35.75	32.215-37.757	4.9	97.9
1:800	17.87	17.35-20.086	4.9	103
1:1600	8.935	6.117-10.48	18	81.1

5. Recovery

The recovery of CA19-9 spiked to 4 different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average Recovery %	Range %
Sample diluent	83.0	80-88
Plasma	111.3	99 – 119

6. Sensitivity

The minimum detectable dose of CA19-9 was determined by adding three standard deviations to the mean optical density value of 16 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of this human CA19-9 using a standard curve generated with Calibrator Diluent was *1.71U/mL.*

7. Specificity

This ELISA kit shows no cross-reactivity with the following recombinant human cytokines and other proteins: IL-8, IL-1 alpha, IL-1beta, IL-6, IL-2, IL-4, IL-10, IL-13, IL-22, IL-17A, IL-12, IL-18, MCAF, EGF, beta-FGF, GM-CSF, TNF-alpha, IFN-gamma, AFP, CEA, PSA, free-PSA, SAA.

8. Expected Normal Values

76 serum/plasma samples from healthy blood donors were tested with the kit. The CA19-9 concentrations in 75 serum/plasma samples were lower than 38U/mL. The CA19-9 concentration in 1 sample was 54U/mL.