Human CA19-9 OneStep ELISA Kit
(CA19-9 OneStep-ELISA)

Cat. No. EK7028

96 Tests in 8 x 12 divisible strips

Background ScienCell's OneStep Human CA19-9 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human CA19-9 with a 96-well strip plate that is pre-coated with antibody specific for CA19-9. The detection antibody is a HRP conjugated antibody specific for CA19-9. The capture antibody is monoclonal antibody from mouse, and the detection antibody is monoclonal antibody from mouse. The kit is analytically validated with ready to use reagents. To measure Human CA19-9, add standards and samples to the wells, then add the HRP conjugated detection antibody. Wash away the unbounded protein and HRP conjugated detection antibody. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. Upon addition of the substrate, the density of the yellow product is linearly proportional to Human CA19-9 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human CA19-9 in the sample.

Size 96 Tests in 8 x 12 divisible strips

Assay type Sandwich ELISA

Range 10 – 150 U/ml

Sensitivity 3.8 U/ml

Specificity Natural and recombinant human CA19-9

Cross-reactivity This kit is for the quantitation of Human CA19-9 concentrations in Serum. No significant cross-reactivity or interference was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs.

Storage Store at 4°C for 6 months, at -20°C for 12 months.
Avoid multiple freeze-thaw cycles.

Shipping Shipped with gel ice.

Application For quantitative detection of human CA19-9 concentrations in Serum.

Kit components

1. Human CA19-9 Standards (S0~S4): CA19-9 (from natural protein) (0, 10，40，100, 150) U/ml, 0.02M PBS, 20% new-born calf serum, 0.5 mL ×5.

2. 8 x 12 divisible strips, pre-coated.
3. 20x wash buffer concentrate: 15 mL.
4. Biotinylated anti- human CA19-9 antibody: 6 mL.
5. HRP-conjugated anti- human CA19-9 antibody: 6 mL.
6. Controls: CA19-9 (from natural protein), 0.02M PBS, 20% new-born calf serum, 0.5 mL .
7. Color developing reagent A: 7 mL, contains 11 mmol/L H_2O_2.
8. Color developing reagent B: 7 mL, contains 2 mmol/L TMB.
9. Stop solution: 7 mL.
10. Plate seals: 2 pieces.

Materials Required But Not Provided

1. Microplate reader.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multi-channel pipettes are recommended for large amount of samples.
4. Clean tubes and Eppendorf tubes.
5. Deionized or distilled water.
6. 500 mL graduated cylinders

Usage

This product is for research use only. It is not approved for use in humans, animals, or *in vitro* diagnostic procedures.

Protocol for Human CA19-9 ELISA

Notes before you begin

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

Preparation

Sample Preparation and Storage

1. Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
2. Avoid multiple freeze-thaw cycles.
3. Prior to assay, frozen sera should be completely thawed and mixed well.

Note: Grossly hemolyzed samples and chylemia samples are not suitable for use in this assay, so the samples should be centrifugated adequately and no hemolysis or granule was allowed.

Reagent Preparation and Storage

Item	Preparation
All reagents	Bring all reagents to room temperature (20-25°C) for 30 minutes.
20X Wash Buffer Concentrate	Prepare 1X wash buffer by adding 15 ml of Wash Buffer Concentrate to 285 ml deionized or distilled water to prepare 300 mL of Wash Buffer.

Assay Procedure

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Take Biotinylated antibody according to 1/10 volume of enzyme conjugate, and mix it with the enzyme conjugate to prepare the mixture. For example, add 0.3ml Biotinylated antibody into 3ml enzyme conjugate to prepare the enzyme conjugate mixture.
4. Set Standard wells, Sample wells, Control wells and Blank wells, add 50 µl of the standard, sample, or control per well. At least two replicates of each standard, sample, control or blank is recommended.
5. Add 50 µl of the enzyme conjugate mixture to each well except for the blank well and mix thoroughly.
6. Cover with plate sealer and incubate for 60 minutes at 37°C.
7. Wash the plate 3 times with the 1x wash buffer.
   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
   c. Repeat steps a-b 2 additional times.
8. Add 50 µl Color Developing Reagent A and 50µl Color Developing Reagent B to each well and incubate in the dark for 15 minutes at 37°C.
9. Add 50 µl of Stop Solution to each well.
10. Read absorbance on Plate Reader at 450 nm within 15 minutes after adding the stopping solution.

Calculation of Results

Average the duplicate readings for each standard, sample, and control. Subtract the average blank O.D. reading.

It is unnecessary to set blank control for dual wavelength plate reader.

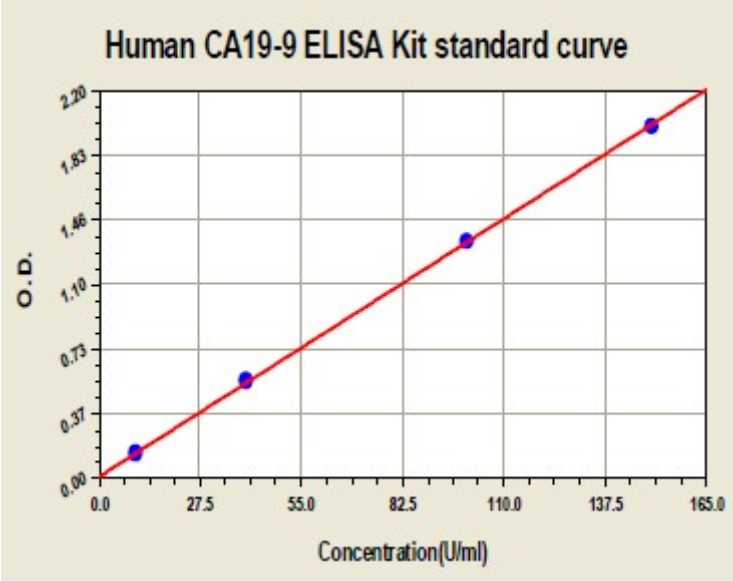
It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. A free program capable of generating a four-parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic- curve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.
For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Typical Data Obtained from Human CA19-9

Concentration (U/ml)	0	10	40	100	150
Absorbance (450 nm)	0.000	0.143	0.561	1.348	1.997

Typical Human CA19-9 ELISA Kit Standard Curve



This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.