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antibody and ELISA experts

PicoKine™ ELISA

Catalog number: EK7029

For the quantitation of **Human CA125** concentrations in Serum.

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

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Human CA125 OneStep ELISA Kit

Catalog Number: EK7029

Introduction

The Bosterbio OneStep Human CA125 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed to quantitatively measure CA125 levels in serum using a sandwich ELISA format based on the streptavidin – biotin system. The assay begins with a microplate pre-coated with streptavidin, forming a solid-phase surface. Add standards or samples separately to the streptavidin pre-coated 96-well strip microplate, then add the antibody mixture. This forms a sandwich complex consisting of streptavidin–biotin-labeled antibody – CA125 – HRP-labeled antibody. Following the addition of substrate and color development, the optical density (OD) is measured using a microplate reader. The CA125 concentration in the samples is calculated by comparing the OD values to a standard curve generated from known concentrations.

Overview

Product Name	Human CA125 OneStep ELISA Kit
Reactive Species	Human
Size	96wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human CA125. 96wells/kit, with removable strips.
Sensitivity	3.8 U/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	15-250 U/ml
Storage Instructions	Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles(Shipped with wet ice.)
Uniprot ID	Q8WXI7
Specificity	Natural and recombinant Human CA125
Cross Reactivity	There is no detectable cross-reactivity.

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Kit Components/Materials Provided

Description	Quantity	Volume	Buffers
Streptavidin Pre-coated 96-well strip microplate	1	8 strips of 12 wells	Polystyrene micro-well plate, pre-coated with streptavidin
Human CA125 Standards(S0~S4)	5	0.5ml	CA125 (from human adenocarcinoma tissue) (0, 15，40，100, 250) U/ml, 0.02M PBS, 20% new-born calf serum
HRP Conjugated anti-Human CA125 antibody	1	6ml	HRP Conjugated anti-Human CA125 antibody, 0.02M PBS, 20% new-born calf serum, 0.01% azophloxine, from mouse monoclonal antibody
Biotin Conjugated anti-Human CA125 antibody	1	6ml	Biotin Conjugated anti-Human CA125 antibody 0.02M PBS, 20% new-born calf serum, 0.01% Evans Blue
20X Wash Buffer Concentrate	1	15ml	Neutral buffer, contains 0.5% Tween-20
Color Developing Reagent A	1	7ml	Contains 11mmol/L H_2O_2
Color Developing Reagent B	1	7ml	Contains 2mmol/L TMB
Stop Solution	1	7ml	2mol/L dilute sulphuric acid
Plate Sealers	2	Piece	

Required Materials That Are Not Supplied

1. Microplate Reader capable of reading absorbance at 450nm.
2. Automated plate washer (optional)
3. Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for large amount of samples.
4. Deionized or distilled water.
5. 500ml graduated cylinders.
6. Test tubes for dilution.

Typical Data Obtained from Human CA125 ELISA Kit

Concentration(U/ml)	0	15	40	100	250
O.D.	0.000	0.226	0.504	1.150	2.283

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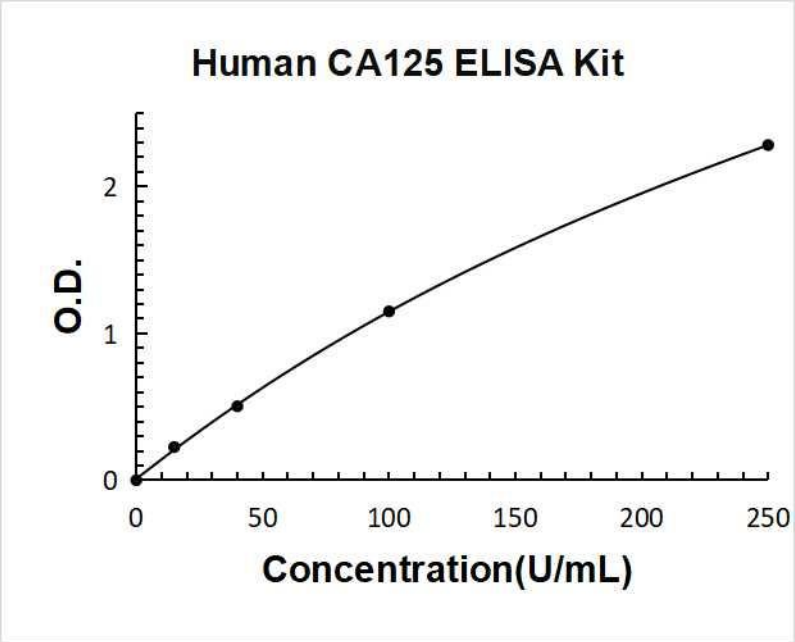

Warnings and Precautions

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.

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.

Preparation Before The Experiment

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. Allow all reagents to equilibrate at room temperature (15–28°C) for half an hour.
2. Before each experiment, prepare the enzyme conjugate working solution by mixing the conjugated antibody with the enzyme conjugate at a 1:10 ratio (volume of conjugated antibody to enzyme conjugate). For example, add 0.3 mL of conjugated antibody to 3 mL of enzyme conjugate and mix thoroughly.
3. Remove the pre-coated plate from the sealed bag. Set up duplicate wells for each calibrator and quality control sample. Add 50 µL of the appropriate calibrator or quality control to each designated well. For test samples, add 50 µL of the test serum directly to the sample wells. Then add 50 µL of the enzyme conjugate working solution to each well (except the blank control well). Mix thoroughly, seal the plate with adhesive film, and incubate at 37°C for 1 hour.
4. Discard the liquid from the wells and fill each well with wash buffer. Let it stand for 10 seconds, then discard the liquid. Repeat this washing process three times, then tap the plate dry on absorbent paper.
5. Add 50 µL of Color Developing Reagent A and 50 µL of Color Developing Reagent B to each well. Mix well by shaking, then incubate at 37°C for 15 minutes in the dark for color development.
6. 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.