Version 26-04/24

User's Manual

Human CA15-3 ELISA Kit

REF **DEIA124J**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Human CA15-3 (Cancer Antigen) ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human CA15-3 in serum, plasma, and cell culture supernatants.

Principles of Testing

This assay employs an antibody specific for human CA15-3 coated on a 96-well plate. Standards and samples are pipetted into the wells and CA15-3 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human CA15-3 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of CA15-3 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents And Materials Provided

1. CA15-3 Microplate (Item A): 96 wells (12 strips × 8 wells) coated with anti-Human CA15-3.
2. Wash Buffer Concentrate (20×) (Item B): 25 ml of 20× concentrated solution.
3. Standard Protein (Item C): 2 vials of Human CA15-3. 1 vial is enough to run each standard in duplicate.
4. Detection Antibody CA15-3 (Item F): 2 vials of biotinylated anti-Human CA15-3. Each vial is enough to assay half the microplate.
5. HRP-Streptavidin Concentrate (Item G): 200 µl 200× concentrated HRP-conjugated streptavidin.
6. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.
7. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.
8. Assay Diluent A (Item D): 30 ml of diluent buffer, 0.09% sodium azide as preservative.
9. Assay Diluent B (Item E): 15 ml of 5× concentrated buffer.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

Storage

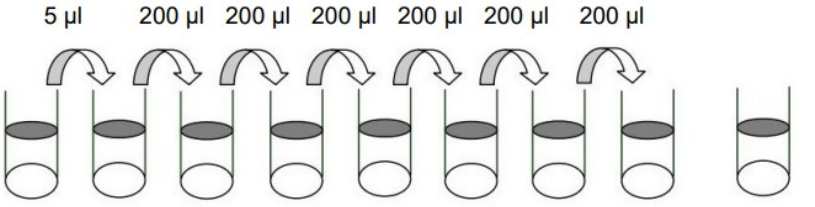
The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage,

1. CA15-3 Microplate: 1 month at 4°C (Return unused wells to the pouch containing desiccant pack, reseal along entire edge.)
2. Wash Buffer Concentrate (20×): 1 month at 4°C
3. Standard Protein: 1 week at -80°C
4. Detection Antibody CA15-3: 5 days at 4°C
5. HRP-Streptavidin Concentrate: Do not store and reuse.
6. Assay Diluent B: 1 month at 4°C

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use.
3. Sample dilution: Assay Diluent A (Item D) should be used for dilution of serum and plasma samples. 1× Assay Diluent B (Item E) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 30 - 200 fold.

   Note: Levels of CA15-3 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
4. Preparation of standard: Briefly spin a vial of Item C. Add 400 µl Assay Diluent A (for serum/plasma samples) or 1× Assay Diluent B (for cell culture supernatants) into Item C vial to prepare a 100 U/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 5 µl CA15-3 standard (100 U/ml) from the vial of Item C, into a tube with 495 µl Assay Diluent A or 1× Assay Diluent B to prepare a 1,000 mU/ml standard solution. Pipette 300 µl Assay Diluent A or 1× Assay Diluent B into each tube. Use the 1,000 mU/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1× Assay Diluent B serves as the zero standard (0 mU/ml).



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C + 400 µl	495 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl		300 µl
Conc.	100 U/ml	1000 mU/ml	400 mU/ml	160 mU/ml	64 mU/ml	25.6 mU/ml	10.24 mU/ml	4.1 mU/ml		0 mU/ml

5. If the Wash Concentrate (20×) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1× Wash Buffer.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1× Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1× Assay Diluent B (Item E) and used in step 5 of Part VI Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 200-fold with 1× Assay Diluent B (Item E).

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 50 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1× Assay Diluent B to prepare a 200-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Assay Procedure

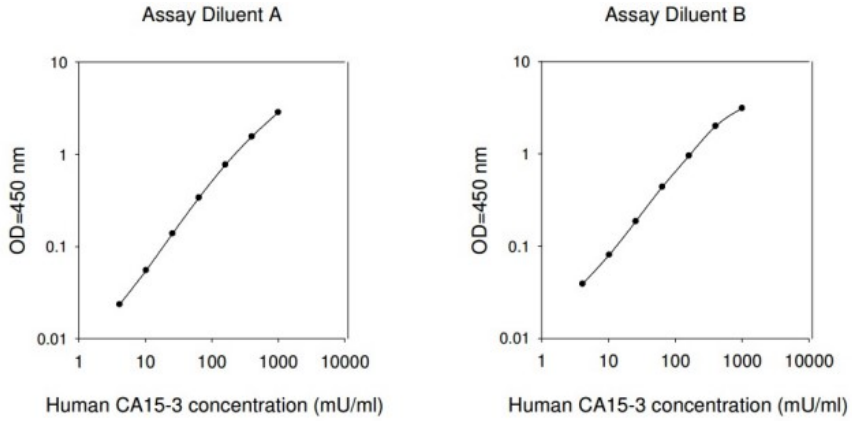
1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µl of each standard (see Reagent Preparation step 3) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1× Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µl of 1× prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Calculation

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using professional plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Standard Curve

These standard curves are for demonstration only. A standard curve must be run with each assay.



Precision

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

Sensitivity

The minimum detectable dose of Human CA15-3 was determined to be 4 mU/ml. Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Specificity

This ELISA kit shows no cross-reactivity with the following cytokines tested: human Angiogenin, BDNF, BLC, CNTF, ENA- 78, FGF-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, FGF-4, FGF-6, FGF- 7, G-CSF, GDNF, GM-CSF, IFN-gamma, IGFBP-2, IGFBP-3, IGFBP-4, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIF, MIG, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, PDGF, RANTES, SCF, SDF-1 alpha, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.

Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	93.03	98.78	96.45
	Range (%)	85-101	91-107	88-104
1:4	Average % of Expected	79.01	98.77	121.0
	Range (%)	71-87	78-138	110-132

Recovery

Recovery was determined by spiking various levels of Human CA15-3 into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	102.9	95-106
Plasma	107.6	100-115
Cell culture media	92.80	85-97

Limitations

Problem	Cause	Solution
Poor standard curve	• Inaccurate pipetting • Improper standard dilution	• Check pipettes • Briefly centrifuge Item C and dissolve the powder thoroughly by gently mixing
Low signal	• Improper preparation of standard and/or biotinylated antibody • Too brief incubation times • Inadequate reagent volumes or improper dilution	• Briefly spin down vials before opening. Dissolve the powder thoroughly. • Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background). • Check pipettes and ensure correct preparation
Large CV	• Inaccurate pipetting • Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Stop solution	• Store your standard at <-70ºC after reconstitution, others at 4ºC. Keep substrate solution protected from light. • Add stop solution to each well before reading plate