Product Manual

Human Cancer Antigen 15- 3 (CA 15- 3) ELISA Kit

Catalog Numbers

PRB- 5069	96 assays
PRB- 5069- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Breast cancer is one of the most commonly diagnosed cancer in women. According to the American Cancer Society, over 250,000 new cases of invasive breast cancer will be diagnosed in 2017. Although death rates from breast cancer have steadily dropped, screening and early-stage detection are key to successful treatment.

CA 15-3, also known as mucin 1, is a membrane-associated glycoprotein often overexpressed in patients with various tumors (ovarian, colon, lung, liver). However, CA 15-3 is most commonly associated with breast cancer and utilized as a biomarker. CA 15-3 is often used to monitor therapy effectiveness and post-operative cancer recurrence (in conjunction with CA 27.29).

Cell Biolabs' Cancer Antigen 15-3 ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the CA 15-3 protein. The kit has detection sensitivity limit of 4 U/mL CA 15-3. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and CA 15-3 samples.

Assay Principle

An anti-CA 15-3 coating antibody is adsorbed onto a microtiter plate. CA 15-3 protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-CA 15-3 antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a streptavidin-enzyme conjugate is added and binds to the biotinylated anti-CA 15-3 antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of CA 15-3 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified CA 15-3 and sample concentration is then determined.

Related Products

1. PRB-5049-C: Human PSA ELISA Combo Kit (Free & Total)
2. PRB-5058: Human AFP ELISA Kit
3. PRB-5059: Human CEA ELISA Kit
4. PRB-5060: Human HE4 ELISA Kit
5. PRB-5061: Human CA 125 ELISA Kit
6. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
7. CBA-110: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric)
8. CBA-125: Radius™ 24-Well Cell Migration Assay
9. CBA-126: Radius™ 96-Well Cell Migration Assay
10. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-CA 15-3 Antibody Coated Plate (Part No. 50691B): One strip well 96-well plate.
2. Biotinylated Anti-CA 15-3 Antibody (1000X) (Part No. 50692D): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human CA 15-3 Standard (Part No. 50693D): One 100 µL vial of 20 kU/mL CA 15-3.

Materials Not Supplied

1. CA 15-3 Sample: serum, plasma, lysate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store CA 15-3 Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-CA 15-3 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-CA 15-3 Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of CA 15-3 Standard in the concentration range of 250 U/mL – 3.91 U/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	20 kU/mL Human CA 15-3 Standard (µL)	Assay Diluent (µL)	CA 15-3 (U/mL)
1	12	948	250
2	500 of Tube #1	500	125
3	500 of Tube #2	500	62.5
4	500 of Tube #3	500	31.25
5	500 of Tube #4	500	15.63
6	500 of Tube #5	500	7.81
7	500 of Tube #6	500	3.91
8	0	500	0

Table 1. Preparation of CA-15-3 Standard

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Add 100 µL of CA 15-3 sample or standard to the Anti-CA 15-3 Antibody Coated Plate. Each CA 15-3 sample, standard, blank, and control should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
4. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-CA 15-3 Antibody to each well.
6. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
7. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 4 above.
8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
9. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
10. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 4 above. Proceed immediately to the next step.
11. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

12. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical CA 15-3 ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

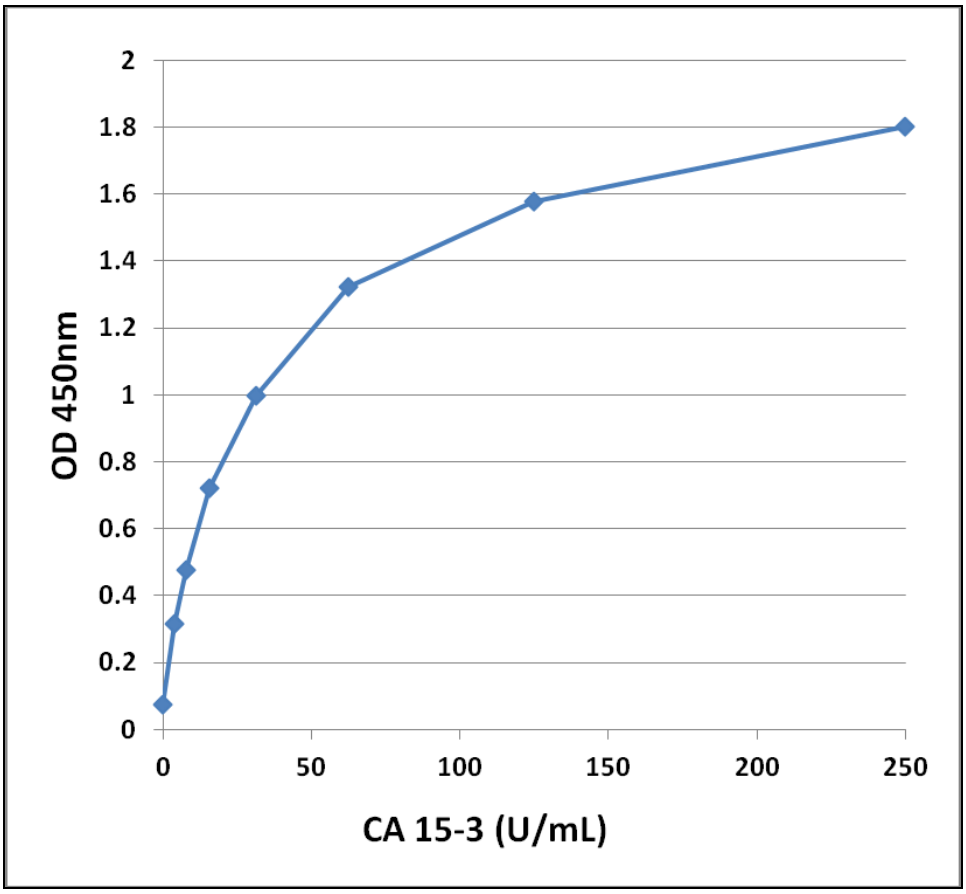

Figure 1: CA 15-3 ELISA Standard Curve

References

1. Luftner, D. et al. (2004) *J. Biol. Markers* **19(3)**:175-182.
2. Nishimura, R. et al. (2003) *Breast Cancer* **10(3)**:220-227.
3. Duffy, M.J. et al. (2004) *Clin. Chem.* **50**:559-565.
4. Bearz, A. et al. (2007) *Int. J. Biol. Markers* **22(4)**:307-311.

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