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Product Manual

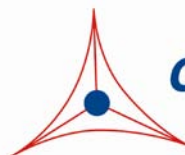
# Human Cancer Antigen 125 (CA 125) ELISA Kit

## Catalog Numbers

PRB- 5061	96 assays
PRB- 5061- 5	5 x 96 assays

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures

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**CELL BIOLABS, INC.**  
*Creating Solutions for Life Science Research*

## **Introduction**

Ovarian cancer causes more deaths than any other cancer of the female reproductive system. This cancer often goes undetected until it has spread within the pelvis and abdomen, making it more difficult to treat and often fatal. Early-stage detection and diagnosis is key to confining the disease to the ovary, making it much more treatable.

Cancer Antigen 125 (CA 125) is the most popular biomarker for ovarian cancer detection. CA 125, also known as mucin 16, is the product of the *MUC16* gene and is often overexpressed in patients with ovarian carcinoma. Cancer Antigen 125 is a membrane-associated glycoprotein shown to participate in tumorigenesis and tumor metastasis. Elevated serum levels of CA 125 are present in ~90% of women with advanced ovarian cancer; additionally, HE4 is often tested in conjunction with CA 125. Combined determination of serum HE4 and CA125 biomarkers is a valuable tool for the diagnosis and monitoring of epithelial ovarian cancer.

Cell Biolabs' Cancer Antigen 125 ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the CA 125 protein. The kit has detection sensitivity limit of 2 U/mL CA 125. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and CA 125 samples.

## **Assay Principle**

An anti-CA 125 coating antibody is adsorbed onto a microtiter plate. CA 125 protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-CA 125 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 125 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 125 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 125 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-5069: Human CA 15-3 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-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

## **Kit Components**

### **Box 1 (shipped at room temperature)**

1. Anti-CA 125 Antibody Coated Plate (Part No. 50611B): One strip well 96-well plate.
2. Biotinylated Anti-CA 125 Antibody (1000X) (Part No. 50612D): One 20  $\mu$ L vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20  $\mu$ L 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 125 Standard (Part No. 50613D): One 100  $\mu$ L vial of 10 kU/mL CA 125.

## **Materials Not Supplied**

1. CA 125 Sample: serum, plasma, lysate
2. 10  $\mu$ L to 1000  $\mu$ L adjustable single channel micropipettes with disposable tips
3. 50  $\mu$ L to 300  $\mu$ L 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 receipt, aliquot and store CA 125 Standard at  $-20^{\circ}\text{C}$  and avoid freeze/thaw. Store all other components at  $4^{\circ}\text{C}$ .

## **Preparation of Reagents**

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-CA 125 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-CA 125 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 125 Standard in the concentration range of 100 U/mL – 1.56 U/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	10 kU/mL Human CA 125 Standard (µL)	Assay Diluent (µL)	CA 125 (U/mL)
1	10	990	100
2	500 of Tube #1	500	50
3	500 of Tube #2	500	25
4	500 of Tube #3	500	12.5
5	500 of Tube #4	500	6.25
6	500 of Tube #5	500	3.13
7	500 of Tube #6	500	1.56
8	0	500	0

**Table 1. Preparation of CA-125 Standard**

### **Assay Protocol**

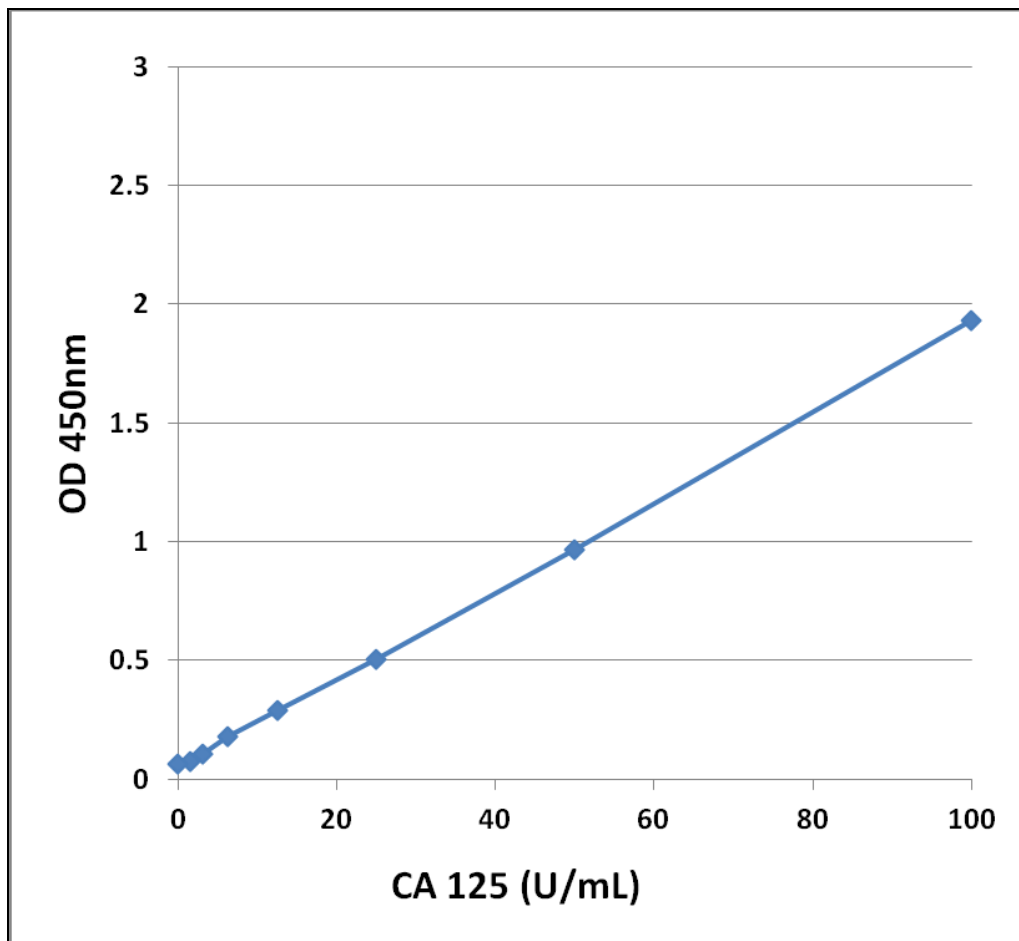
1. Prepare and mix all reagents thoroughly before use.
2. Add 100 µL of CA 125 sample or standard to the Anti-CA 125 Antibody Coated Plate. Each CA 125 sample, standard, blank, and control should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital 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 125 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  $\mu$ L 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 125 ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.



**Figure 1: CA 125 ELISA Standard Curve**

### **References**

1. Kaneko, O. et al. (2009) *J. Biol. Chem.* **284**:3739-3749.
2. Rump, A. et al. (2004) *J. Biol. Chem.* **279**:9190-9198.
3. Shimizu, A. et al. (2012) *Cancer Sci.* **103**:739-746.

## **Warranty**

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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