Product Manual

Human Epididymis Protein 4 (HE4) ELISA Kit

Catalog Numbers

PRB- 5060	96 assays
PRB- 5060- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Ovarian cancer causes more deaths than any other cancer of the female reproductive system. This cancer often goes undetected until it's 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.

Human Epididymis Protein 4 (HE4), a relatively new tumor marker, is the product of the *WFDC2* gene and often overexpressed in patients with ovarian carcinoma. The exact molecular weight of natural HE4 is not yet known (roughly 25-35 kDa) and it's biological role is unclear. However, elevated serum levels are detected in early stages of tumor progression and in patients with normal levels of CA 125 (another ovarian cancer marker with lower specificity than HE4). Combined determination of serum HE4 and CA 125 biomarkers is a valuable tool for the diagnosis and monitoring of epithelial ovarian cancer.

Cell Biolabs' HE4 ELISA Kit is an enzyme immunoassay developed for detection and quantitation of HE4 protein. The kit has detection sensitivity limit of 1 pg/mL HE4. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and HE4 samples.

Assay Principle

An anti-HE4 coating antibody is adsorbed onto a microtiter plate. HE4 protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-HE4 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-HE4 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 HE4 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 HE4 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-5061: Human CA 125 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-HE4 Antibody Coated Plate (Part No. 50601B): One strip well 96-well plate.
2. Biotinylated Anti-HE4 Antibody (1000X) (Part No. 50602D): 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. Recombinant HE4 Standard (Part No. 50603D): One 100 µL vial of 50 ng/mL HE4.

Materials Not Supplied

1. HE4 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 receipt, aliquot and store HE4 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-HE4 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-HE4 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 HE4 Standard in the concentration range of 50 pg/mL – 0.78 pg/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	50 ng/mL Recombinant HE4 Standard (µL)	Assay Diluent (µL)	HE4 (pg/mL)
1	4	3996	50
2	500 of Tube #1	500	25
3	500 of Tube #2	500	12.5
4	500 of Tube #3	500	6.25
5	500 of Tube #4	500	3.13
6	500 of Tube #5	500	1.56
7	500 of Tube #6	500	0.78
8	0	500	0

Table 1. Preparation of HE4 Standard

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Add 100 µL of HE4 sample or standard to the Anti-HE4 Antibody Coated Plate. Each HE4 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-HE4 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 HE4 ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

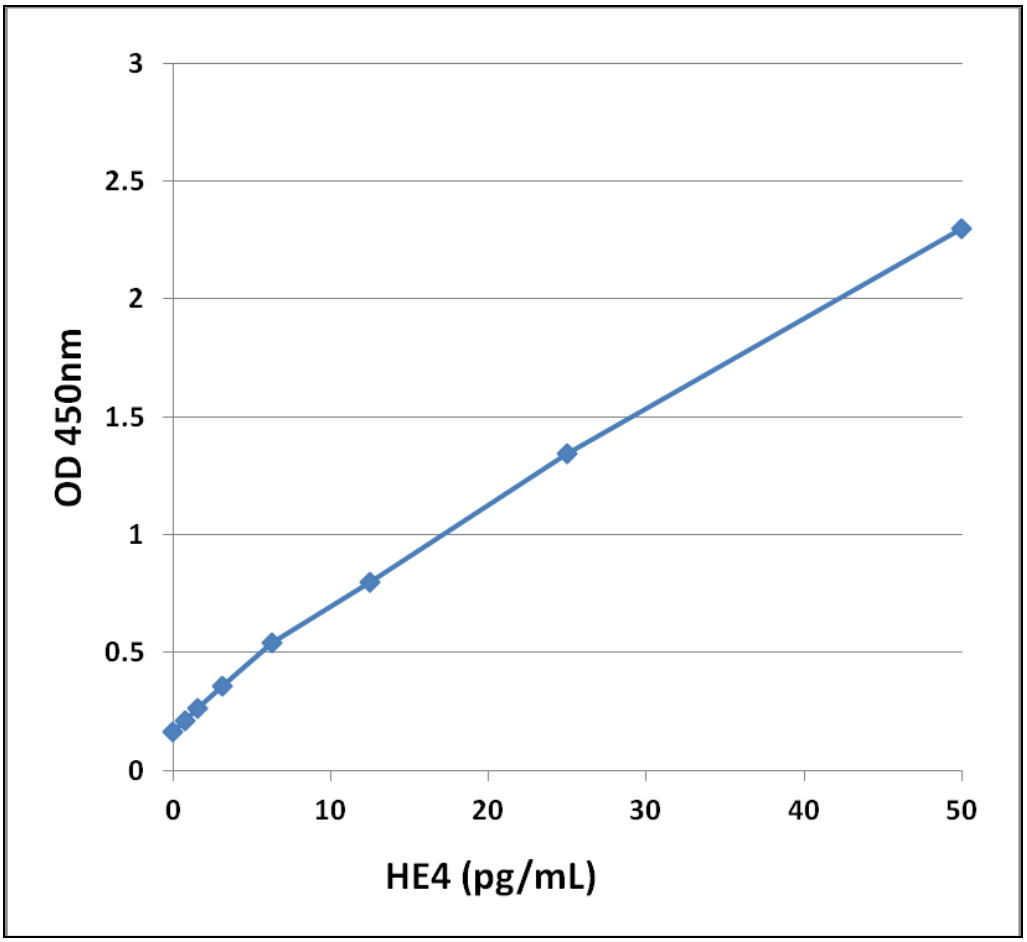

Figure 1: HE4 ELISA Standard Curve

References

1. Bingle, L. et al. (2002) *Oncogene* ***21****:2768-2773.*
2. Drapkin, R. et al. (2005) *Cancer Res.* ***65****:2162-2169.*
3. Galgano, M. et al. (2006) *Mod. Pathol.* ***19****:847-853.*

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.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com