Product Manual

Human Total PSA (t-PSA) ELISA Kit

Catalog Numbers

PRB-5049-TOTAL	96 assays
PRB-5049-TOTAL-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Prostate-Specific Antigen (PSA) is a 33 kDa serine proteinase secreted by epithelial cells of the prostate gland. PSA exists in serum in 3 main forms: unbound free PSA (f-PSA), α_1-antichymotrypsin bound PSA, and α_2-macroglobulin bound PSA. Blood PSA levels are typically low in men with healthy prostates, but are often elevated in individuals with prostate disorders (i.e. cancer, prostatitis, benign prostate hyperplasia). Measuring the ratio of free to total PSA (t-PSA) is an effective tool in prostate cancer screening and diagnosis.

Cell Biolabs' Total PSA ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the human PSA protein. The kit has a detection sensitivity limit of 15 pg/mL and contains a HYBRITECH® PSA Calibration Standard. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and PSA samples.

Note: This kit detects bound and unbound forms of PSA.

Assay Principle

An anti-t-PSA coating antibody is adsorbed onto a microtiter plate. PSA protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-t-PSA 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-t-PSA 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 PSA 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 a PSA calibrator and sample concentration is then determined.

Related Products

1. PRB-5033: Human Alpha 2 Macroglobulin ELISA Kit
2. PRB-5039: Human Haptoglobin ELISA Kit
3. PRB-5041: Human Ceruloplasmin ELISA Kit
4. PRB-5044: Human Alpha 1 Antitrypsin ELISA Kit
5. PRB-5047: Human CK-MB ELISA Kit
6. PRB-5049-C: Human PSA ELISA Combo Kit (Free + Total PSA)
7. PRB-5049-FREE: Human Free PSA ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-t-PSA Antibody Coated Plate (Part No. 50494B): One strip well 96-well plate.
2. Biotinylated Anti-t-PSA Antibody (1000X) (Part No. 50495D): 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 t-PSA Standard (Part No. 50496B): One 100 µL vial of 50 ng/mL human PSA (HYBRITECH® Calibration Standard).

   Note: For WHO Calibration values, this standard is equivalent to 40 ng/mL.

Materials Not Supplied

1. PSA 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, store all components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-t-PSA Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-t-PSA 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 t-PSA Standard in the concentration range of 1 ng/mL – 0.016 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	50 ng/mL HYBRITECH® t-PSA Standard (µL)	Assay Diluent (µL)	HYBRITECH® t-PSA (ng/mL)
1	16	784	1
2	400 of Tube #1	400	0.5
3	400 of Tube #2	400	0.25
4	400 of Tube #3	400	0.125
5	400 of Tube #4	400	0.063
6	400 of Tube #5	400	0.031
7	400 of Tube #6	400	0.016
8	0	400	0

Table 1. Preparation of t-PSA Standard

Assay Protocol

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

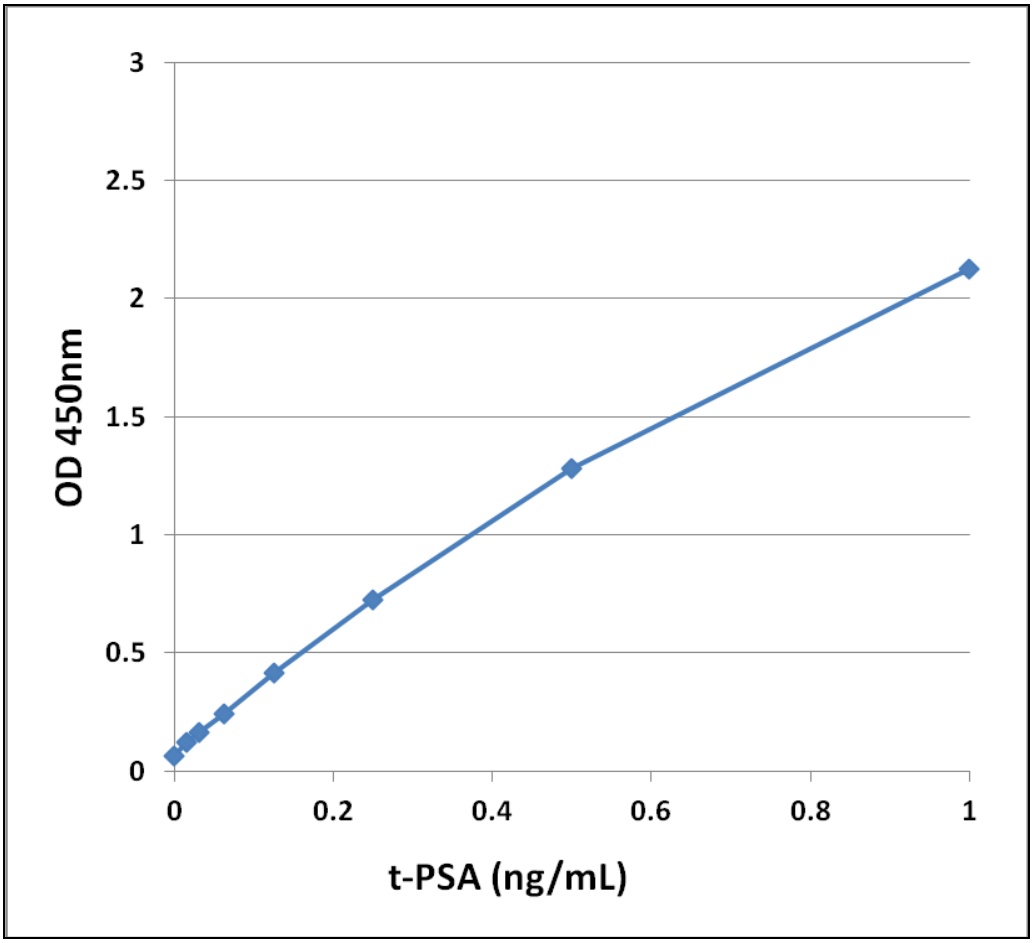

Figure 1: Total PSA ELISA Standard Curve

References

1. Christensson, A. et al. (1993) *J. of Urol.* **150**:100-105.
2. Lilja, H. et al. (1991) *Clin. Chem.* **37**:1618-1625.
3. Stamey, T. et al. (1987) *N. Engl. J. Med.* **317**:909-916.

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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