General References

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(2) Citations of ADI's PSA ELISA kit (see web site for updated list)

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Kim J-H	2003	Clin. Cancer Res., 9: 4782 - 4791. A PSA ELISA kit was used to measure PSA in the medium of LNCaP cells cells.
Zhao H	2004	Mol. Biol. Cell, 15: 506 - 519. PSA in culture medium
Sreekumar A	2004	J Natl Cancer Inst, 96: 834–843, PSA and CEA.
Kyprianou D	2009	Biosensors and Bioelectronics, 24, 1365-1371 "
Lin Y-Y	2008	Biosensors and Bioelectronics, 23, 1659-1665
Kyprianoua D	2008	Biosensors and Bioelectronics 24, 1365-1371

Related ELISA kits for other serum proteins

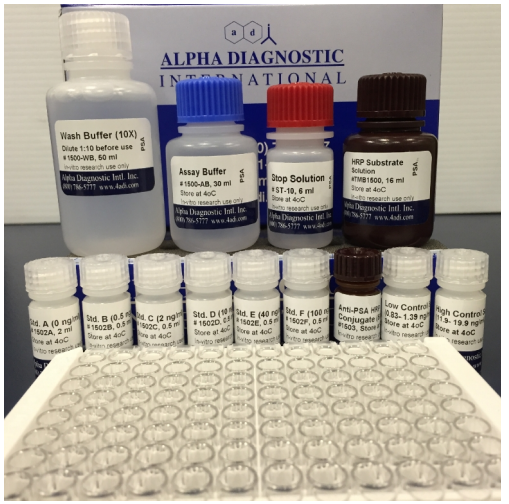
CatalogNumber	ItemName
0050	Human Neuron Specific Enolase (NSE) ELISA Kit
1750	Human IgG (total) ELISA Kit
1760	Human IgM ELISA Kit
1800	Human IgE ELISA Kit
0410	Human Chorionic Gonadotropin (HCG-free beta) ELISA Kit
0040	Human C-peptide ELISA Kit
0200	Human Folicle Stimulating Hormone (FSH) ELISA Kit
0100	Human Luteinizing Hormone (LH) ELISA Kit
0030	Human Insulin ELISA Kit
0300	Human Prolactin (PRL) ELISA Kit
0400	Human Chorionic Gonadotropin (HCG) ELISA Kit
0500	Human Alpha Fetoprotein (AFP) ELISA Kit
1000	Human C-Reactive Protein (CRP) ELISA Kit
1200	Human Albumin ELISA Kit
0020	Human Beta-2 microglobulin (B2M) ELISA Kit
1810	Human Ferritin ELISA Kit
0700	Human Sex Hormone Binding Glob (SHBG) ELISA Kit
1210	Human Transferrin (Tf) ELISA Kit
0010	Human Leptin ELISA Kit
200-120-AGH	Human globular Adiponectin (gAcrp30) ELISA Kit
1190	Human Serum Albumin ELISA Kit
100-140-ADH	Human Adiponectin (Acrp30) ELISA Kit
100-110-RSH	Human Resistin /FIZZ3 ELISA Kit
1840	Human Pancreatic & GI Cancer (CA199) ELISA Kit
1830	Human Ovarian Cancer (CA153) ELISA Kit
1310	Human Pancreatic Lipase ELISA Kit
1600	Human Growth Hormone (GH) ELISA Kit
1510	Human Free Prostate Specific Antigen (PSA-free) ELISA Kit
1500	Human Prostate Specific Antigen (PSA) ELISA Kit
1400	Human Prostatic Acid Phosphatase (PAP) ELISA Kit
0900	Human IGF-Binding Protein 1 (IGFBP1) ELISA Kit
0060	Human Pancreatic Colorectal cancer (CA-242) ELISA Kit
1820	Human Ovarian Cancer (CA125) ELISA Kit

Instruction Manual No. M-1500

Human Prostate Specific Antigen (PSA)

ELISA KIT Cat. No. 1500

For Quantitative Determination of Human PSA In Serum



For In Vitro Research Use Only

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Human Prostate Specific Antigen (PSA) ELISA KIT # 1500

Kit Contents: (reagents for 96 tests)

Components	qty
Anti-human PSA coated microwell strip plate (96 wells), #1501	1 plate
PSA **Standard A** (0.0 ng/ml), 2 ml, #1502A	1 bottle
PSA **Standard B** (0.5 ng/ml), 0.5 ml, #1502B	1 vial
PSA **Standard C** (2.0 ng/ml) 0. 5 ml #1502C	1 vial
PSA **Standard D** (10.0 ng/ml), 0. 5 ml , #1502D	1 vial
PSA **Standard E** (40 ng/ml), 0. 5 ml, #1502E	1 vial
PSA **Standard F** (100 ng/ml), 0. 5 ml, #1502F	1 vial
PSA **Low** (#1500-LC) & **High Controls** (#1500-HC) lot sp. values printed on vials; 0.5 ml each	
Anti-PSA IgG-HRP Conj. (50X); 0.3 ml ; Dilute 1 :50 with assay buffer, #1503	1 vial
Assay Buffer 30 ml, #1500-AB	1 bottle
Wash buffer (10X), 50 ml (dilute 1:10 with distilled water), #1500-WB	1 bottle
HRP substrate Solution, 16 ml, # TMB1500	1 bottle
Stop solution (ready-to-use), 6 ml ST-10	1 bottle
Complete Instruction Manual	M-1500

Introduction

Human Prostate-specific antigen (PSA) is a single chain glycoprotein with Mol. Wt. Of 34 kDa containing 7% carbohydrates. It is uniquely associated with prostate tissue and presents in both normal and cancerous prostatic tissue, prostatic fluid and seminal plasma (1). PSA is not present in any of the normal tissue obtained from men nor is it produced by cancers of the lung, colon, rectum, stomach, breast, pancreas or thyroid (2).

Elevated serum PSA concentration have been reported in patient with prostate cancer, benign prostatic hypertrophy, or inflammatory tissues, but not in apparently healthy women with cancer (3). PSA levels correlate with the stage of disease and response to treatment in-patients with prostatic cancer (4-7). Therefore sequential measurements of PSA concentration can be an important tool in monitoring patients with prostatic cancer and in determining the potential and effectiveness of surgery of other therapies.

ADI's PSA ELISA kit provides for the measurement of PSA in serum for monitoring patients with prostatic cancer.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicate determinations of the zero standard, the minimum concentration of human PSA detected using this assay is 0.1 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Sample	Mean (ng/ml)	SD	CV%
1	5.59	0.02	6.1
2	22.89	0.06	4.6

Inter-assay precision:

Sample	Mean (ng/ml)	SD	CV%
1	0.92	0.09	9.5
2	3.64	0.14	3.8

3. RECOVERY

A known amount of hPSA was added to three patient sera (with original PSA concentrations of (2.72, 7.0, and 14.26 ng/ml)) and the total PSA concentrations measured. The assay showed excellent mean recoveries of about 96% (range 94-114%).

4. **Linearity of Dilution**

Three samples were serially diluted (1:2-1:8) with calibrator A and total PSA measured (recovery 92-112%).

5. **High dose hook effect**: No hook effect was observed with PSA at 1000 ng/ml.

6. Comparative Studies

ADI PSA ELISA kit(y) was measure with a competitor (X) using 168 samples. An excellent correlation was observed: y=0.792x+0.8551; r=0.9837

5. SPECIFICITY

The specificity of PSA kit was determined by measuring interference from high concentrations of human serum proteins, hormones, and tumor markers. No cross reactivity was observed. The human PSA ELISA kit #1500 detects total PSA. ADI has another ELISA kit #1510 for free PSA. Antibodies used in the kit are specific for PSA with no reactivity with other tumor antigens or serum proteins.

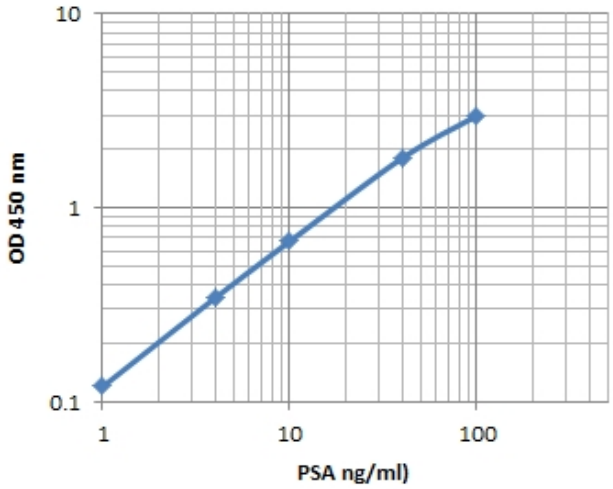
Species cross reactivity and use of other biological fluids

Human PSA kit has not been tested in other species (mouse, rat, monkey etc). The kit may work in monkey samples but the reactivity with other animals is less likely. Human PSA kit has been validated for use in serum. However, it has been used to detect PSA released into culture medium by some cancer cells (LnCap etc). It is recommended to include fresh culture media to establish baseline values or any crossreactivity due to serum components. See published references on page 7.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Mean $A_{450\ nm}$	Calculated Conc. (ng/ml)
A1, A2	**Std. A** (0)	0.093	
B1, B2	**Std. B** (0.5)	0.160	
C1, C2	**Std. C** (2)	0.344	
D1, D2	**Std. D** (10)	0.673	
E1, E2	**Std. E** (40)	1.908	
F1, F2	**Std. F** (100)	2.830	
G1, G2	**Sample 1**	0.341	2.01

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



Kit-specs-XL

A typical std. curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate PSA concentrations. Read off the PSA concentrations of the control and patient samples. If ELISA reader software is being used, we recommend 4-paramter or 5-parameter curve. Samples reading >100 ng/ml should be diluted and re-tested.

PRINCIPLE OF THE TEST

PSA ELISA kit is based on simultaneous binding of human prostate specific antigen (PSA) from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of PSA present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of PSA in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (10-200 ul) and multichannel pipet with disposable plastic tips, Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic International PSA ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20^{o}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Stability of Samples

PSA is unstable at 4oC and 20oC. Samples should not be stored at room temp or 4oC for more than 24 hours. It is recommended to freeze all samples as soon as possible. Avoid repeated freeze and thaw.

Preparation of the reagents:

Dilute wash buffer (1:10) with distilled water (50 ml stock in total of 450 ml water). Store at 4oC.

Dilute enzyme conjugate 1:50 (eg; 20 ul of HRP in 1 ml of assay buffer or 200 ul in 20 ml for a full plate assay). Prepare as needed and do not store diluted conjugate.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. Do not expose these HRP solutions to strong light during storage or use. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE - *ALLOW **ALL** REAGENTS TO REACH ROOM TEMPERATURE (25-30oC) BEFORE USE. Addition of cold reagents will reduce reaction rate and less color).* **Dilute wash buffer (1:10) with distilled water (50 ml stock in 450 ml). Dilute enzyme conjugate 1:50 before use.**

1. Remove required # strips and arrange them on the ELISA frame. Any used strips can be stored in the supplied plastic bag with the desiccant at 4oC. The ELISA plate frame can be saved after the test to be used again if partial plate was used for the assay.

2. Label or mark the microtiter well strips to be used on the plate. Pipet **25 ul of standards, control, and serum samples** into appropriate wells in *duplicate*.

3. Add **100 ul of assay buffer** into each well**.** Mix gently for 5-10 seconds. Cover the plate and **incubate on a plate shaker** (approx. 200 rpm) for **60 min** at room temp (25-28oC).

4. Aspirate and wash the wells **3 times with 300 ul** of 1X wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

5. Add **100 ul Ab-enzyme conjugate buffer** into each well**.** Mix gently for 5-10 seconds. Cover the plate and **incubate on a plate shaker** (approx. 200 rpm) for **30 min** at room temp.

6. Aspirate and wash the wells **3 times with 300 ul** of 1X wash buffer.

7. Dispense **100 ul TMB substrate per well.** Mix gently. Cover the plate and incubate on a plate shaker (approx. 200 rpm) for **15 minutes** at room temperature until dark blue or A450=2.5-3.0.

8. Stop the reaction by adding **50 ul** of stopping solution to **all wells** at the same timed intervals as in step 6. Mix gently.

9. Measure the absorbance at 450 nm using an ELISA reader. If the A450 of the upper standard exceeds the detection limit then the final incubation time at step 7 can be reduced by about 5 minutes. Alternatively, plates can be read at 405-415nm filter, the absorbance will be lower but it will not impact the results.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C.

Addition of the HRP substrate solution starts a kinetic reaction. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Serum samples reassayed, and the results obtained should be multiplied by the appropriate dilution factor if samples were diluted..

EXPECTED VALUES

1. It is recommended that each laboratory must determine its own normal and abnormal ranges. Serum samples from normal subjects (N = 62) were assayed and 98% of the samples were found to have PSA concentrations of less than 2.5 ng/ml. However, the levels of normal reference values are age-related; in the studies of older age groups, 3% of the value in the 40 to 49 age group has been found to be up to 4 ng/mL, and 3.2% of the value in the 50-79 age group, were 4.5-8.6 ng/mL.
2. Serum samples from 120 patients with benign Hypertrophy (BPH) were assayed and 66% of Serum samples observations were less than 2.5 ng/mL.
3. Serum samples from 30 patients with prostatic carcinoma (PC) were assayed and 87% of observations were higher than 2.5 ng/mL.
4. Elevated PSA levels can be an indication of the presence of prostate cancer, they can also be the result of some other prostate diseases such as BPH; therefore, the test must be used in conjunction with a digital rectal examination (DRE) and that if either test is positive. Confirmatory testing with transrectal ultrasound and biopsy is needed to diagnose prostate cancer. Conversely, low PSA levels do not necessarily indicate an absence of prostate cancer.

Quality Control

Good laboratory practices include the use of appropriate controls to assure the validity of the assay. All controls must fall within the limits provided for controls. Any gross deviations should warrant testing of controls and assay procedures or it may indicate a lot failure.