ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	**#0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-1510

Human Free Prostate Specific Antigen (fPSA)

ELISA KIT Cat. No. 1510

For Quantitative Determination of Human fPSA In Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Web Site: www.4adi.com

Human free Prostate Specific Antigen (fPSA)
For Quantitative Determination of Human fPSA In Serum
Kit Contents: (reagents for 96 tests); **ELISA KIT Cat. No. 1510**

Components	Cat. No.
Anti-fPSA coated microwell strip plate (96 wells), Ready-to-use	1511
fPSA **Standard A**, (0 ng/ml), 11 ml	1512
fPSA **Standard B** (0.15 ng/ml), 0.75 ml	1513
fPSA **Standard C** (0.5 ng/ml), 0.75 ml	1514
fPSA **Standard D** (1.5 ng/ml), 0.75 ml	1515
fPSA **Standard E** (5 ng/ml), 0.75 ml	1516
fPSA **Standard F** (15 ng/ml), 0.75 ml	1517
fPSA **Control serum low & high** (exact values printed on vials)	
biotinylated anti-fPSA and Streptavidin-HRP and Conjugate; 11 ml	1518
HRP substrate Solution; 11 ml	TMB15 10
Wash buffer (100X), 10 ml, dilute 1:100 with distilled water	W-100
Stop solution (ready-to-use), 10 ml	T-10
Complete Instruction Manual	M-1510

Introduction:

Human Prostate-Specific Antigen (PSA) is a protein expressed only in the prostatic secretory epithelium. It is a protease with chymotrypsin-like activity. Its function is to liquefy semen through hydrolysis of semenogelin. PSA occurs in serum in at least three different forms: free PSA (fPSA, MW (molecular weight) 30kDa), PSA bound to alpha-1-anti-chymotrypsin (ACT-PSA), MW 90 kDa. Although A2M is the major binder of PSA in serum, ACT-PSA is the predominant immunoreactive form. fPSA, an enzymatically inactive form, represents a smaller immunoreactive fraction (5-30%) (1,2).

PSA assays have been used as a standard assay for prostate cancer detection. However, elevated serum PSA concentration have been reported in patients not only with prostate cancer, but also benign prostatic hypertrophy or other adjacent genitourinary tissue (3). Therefore, PSA is organ specific, not disease specific. Thus the free to the total PSA ratio was the earliest serum marker indicating a subsequent diagnosis of prostatic cancer (1). Measuring free unbound form of PSA can help physicians decide whether or not a biopsy a patient with high levels of total PSA as well as detect additional cases of cancer in men with “normal” total PSA level.

ADI's **fPSA kit** provides an enzyme linked immunosorbent assay system for the measurement of fPSA in serum. To obtain a correct ratio of fPSA to total PSA, ADI PSA kit must be used in conjunction with this kit. Please be aware that the mix-and-match of total and free PSA assays could result in erroneous values.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on twenty replicate determinations of the zero standard, the minimum concentration of fPSA 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:

Three serum samples were run in ten replicates in an assay. The samples showed good intra-assay precision (6.99-8.9 %CV). The actual values were: mean 0.18 ng/ml, SD 0.01 ng/ml, %CV 7.84; mean 0.96 ng/ml, SD 0.09 ng/ml, %CV 8.9; mean 2.83 ng/ml, SD 0.20 ng/ml, %CV 6.99, respectively

Inter-assay precision:

Three serum samples were run in duplicate in eight independent assays. The samples showed good inter-assay precision (4.86-12.55 %CV). The actual values were: mean 0.14 ng/ml, SD 0.02 ng/ml, %CV 12.55; mean 0.80 ng/ml, SD 0.08 ng/ml, %CV 9.59; mean 2.00 ng/ml, SD 0.10 ng/ml, %CV 4.86, respectively.

3. RECOVERY

A known amount of fPSA (7.5 ng/ml) was added to various std fPSA and the total fPSA concentrations measured. The assay showed excellent mean recoveries of 95-115%.

4. SPECIFICITY

The specificity of fPSA kit was determined by measuring interference from high concentrations of human serum proteins, hormones, and tumor markers. No cross reactivity was observed.

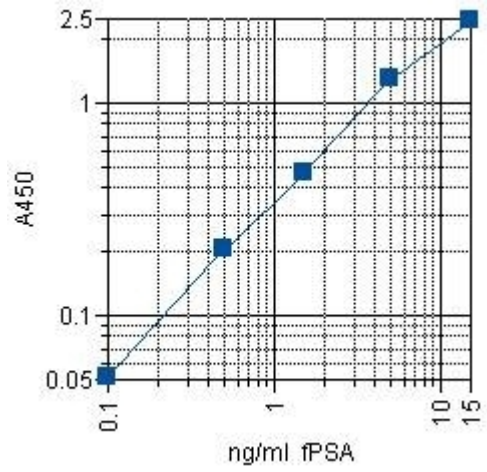
5. SPECIES CROSSREACTIVITY

References: 1. Chen Z et al (1995) Clin Chem. 41, 1273-1285; 2. Van Cang PJ et al (1996) The Prostate Supllement 7, 30-34; 3. Paisdero LD et al (1980) Cancer Res. 40, 2428; 4 Higashihara E et al (1996) Prostate Supplemt 7, 40-47

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (ng/ml)	Mean $A_{450\ nm}$	Net $A_{450\ nm}$	Calculated Conc. (ng/ml)
A1, A2	**Std. A** (0.0)	0.00		
B1, B2	**Std. B** (0.1)	0.051	0.051	
C1, C2	**Std. C** (0.5)	0.206	0.206	
D1, D2	**Std. D** (1.5)	0.469	0.469	
E1, E2	**Std. E** (5)	1.302	1.302	
F1, F2	**Std. F** (15)	2.471	2.471	
G1, G2	**Sample 1**		0.398	1.45

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.



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

PRINCIPLE OF THE TEST

fPSA ELISA kit is based on simultaneous binding of human free prostate specific antigen (fPSA) 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 fPSA 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 fPSA in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µl) 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 Prolcin-300 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).
All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

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.

Reagent Preparation

Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter). Store at 4oC.

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. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions.

HRP substrate buffer (solution A) and HRP substrate (solution B) should be colorless at the time of use. If solutions have turned light blue in color, these should be replaced. Do not expose these solutions to strong light during storage or use. Reconstituted control serum is stable for one week at 2-8^{o}C. 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 BEFORE USE). BEFORE USE. Addition of cold reagents will reduce reaction rate and less color.).* **Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter).**

Add 200-300 ul of 1X wash buffer to all strips that are being used for the assay right before addition of the samples. Manually shake the wells for 5-10 seconds and discard the contents. Tap the plates over paper towels to remove any traces of liquid. This step improves the addition of small volume of samples due to the wet surface of the wells. Do not allow the wells to dry. Immediately start adding the samples and other reagents.

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **50 ul** of standards, control, and serum samples into appropriate wells in *duplicate*.
3. Add **100 ul** of Ab-enzyme conjugate into **each well.** Mix gently.
4. Cover the plate and incubate for **60 minutes** at room temperature.
5. Aspirate and wash the wells **5 times** with 300 ul diluted 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.
6. Dispense **100 ul TMB substrate per well.** Mix gently.
7. Cover the plate and incubate for **30 minutes** at room temperature.
8. Stop the reaction by adding **50 µl** 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.

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 containing more than 10 ng/ml PSA should be diluted with the zero standard (standard A), reassayed, and the results obtained should be multiplied by the appropriate dilution factor.

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 fPSA concentrations. Read off the fPSA concentrations of the control and patient samples.

EXPECTED VALUES

1. It is recommended that each laboratory must determine its own normal and abnormal ranges.
2. It is documented that among men with total PSA level in the 4-10 ng/ml range, the % of fPSA varies from 4-50% (1). A fPSA/tPSA ratio larger than 0.25 indicates a likely presence of BPH; a ratio <0.06 indicates a likely presence of prostate cancer (4).
3. This 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 ratio of free to total PSA do not necessarily indicate an absence of prostate cancer.