Human PSA ELISA Kit

Medical Device Licence No.: 34067

Enzyme immunoassay kit for the quantitative determination of PSA concentration in serum.

Catalog Number: SL100299

96 tests

For *in vitro* diagnostic use only.

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INTENDED USE

ANOGEN's **ELISA-PSA** kit is an enzyme immuno-assay designed for the quantitative measurement of prostatic specific antigen (PSA) in human serum. This *in vitro* device is strictly intended to help in the screening, management and prognosis of patients with prostate cancer at various stage of the disease.

INTRODUCTION

Prostate cancer is the most frequent type of cancer found in men and the second most frequent cause of death due to cancer in men. It responds to treatment better if diagnosed early. Digital rectal examination was the most accepted diagnostic modality for early stage prostate cancer but the measurement of serum PSA has become the most accepted test to indicate men who are at risk of having prostate cancer and who should be examined by other tests, especially ultrasonography of the prostate. Other biochemical markers such as prostatic acid phosphatase, total alkaline phosphatase, carcinoembryonic antigen and creatine kinase isoenyme BB, etc., are not as specific for prostate cancer.

In 1979, Wang *et al.* isolated a specific antigen for normal prostatic tissue and called this protein PSA. PSA is identical to a protein (p30) previously isolated in 1978 by Sensabaugh. Several groups using both amino acid and cDNA sequencing techniques have determined the amino acid sequence of PSA. The consensus sequence is that determined by Lundwall and Lilja and Schaller and co-workers, and later confirmed by Riegman *et al*. This sequence of PSA contains 237 amino acids, with a molecular weight of 26 079 for the peptide moiety of the molecule.

As demonstrated by immunohistological studies, PSA is localized in the cytoplasm of prostatic acinar cells, ductal epithelium and in the secretion on the ductal lumina.

PSA is present in normal, benign hyperplastic and malignant prostatic tissues as well as in metastatic prostate cancer and in seminal plasma. An elevation of the serum PSA concentration has been reported in patients with both benign prostatic hypertrophy and prostatic carcinoma, but rarely in healthy men and is absent in normal women. There is no PSA present in any other normal tissue obtained from men or in patients with other cancers of the breast, lung, colon, rectum, stomach, pancreas or thyroid.

Serum testing for PSA is a very important tool to screen for prostate cancer and to monitor therapy of this disease. Serial measurement of PSA concentration in the serum is an important tool in monitoring patients with prostate cancer and determining the effectiveness of surgery or other therapies and may allow for earlier discovery of residual or recurrent carcinoma after radical prostatectomy or radiotherapy.

PRINCIPLES OF THE ASSAY

ANOGEN's **ELISA-PSA Kit** is a solid phase two-site immunoenzymatic assay, commonly referred to as a "sandwich" assay that uses polyclonal anti-human PSA antibodies (PAb) immobilized on the plastic well as capture reagent and two monoclonal antibodies (MAb) recognizing different PSA epitopes as conjugate. Samples containing PSA react first with coated polyclonal antibodies on plastic well (solid phase). After a washing step, they react

with monoclonal antibodies specific to different epitopes on the same PSA molecule. After the formation of the PAb/PSA/MAb-HRP sandwich, the unbound enzyme conjugate is washed away. The substrate-chromogen solution added is catalyzed by the enzyme Horse Radish Peroxydase (HRP) to form a blue color. The reaction is terminated by adding the stopping solution that turns the blue color to yellow. Since the intensity of the color produced is directly proportional to the PSA concentration, the PSA value in the samples is determined from a standard curve established with PSA standards.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out in accordance with the package insert instructions and with adherence to good laboratory practice.
2. All reagents should be allowed to come to room temperature (22 ± 2°C) prior to beginning the assay. Temperature variation may affect the kinetics of the reaction and lead to variation in results.
3. Keep the incubation times the same for all wells by pipetting the samples and the conjugate without interruption and by adding reagents at timed intervals. To ensure consistency of timing of the assay and to avoid any drift, the size of the assay run should be limited to the number of samples that can be pipetted within 10 minutes.
4. For convenience, repeating pipettors or multichannel dispensers may be used for pipetting reagents and wash solution. To avoid potential sample carry-over, use pipettes with disposable tips for pipetting standards, controls and specimens. Careful decantation or aspiration of the washing solution is essential to the precision of the assay.
5. Avoid strong light or direct sunlight on the plates during the assay.
6. Specimens containing greater than 50 µg/L of PSA should be diluted with the Zero Standard solution and re-assayed. The dilution factor must be incorporated into the calculation of results. The recommended dilutions for samples are 1/5, 1/10. It is desirable to dilute the serum samples that contain more than 50 µg/L of PSA in a way so that the diluted sample can be read greater than the lowest standard and close to the range of the standard curve corresponding to an O.D. about 1.0.
7. Samples with extremely high concentrations may paradoxically read within the standard range of the assay and, after dilution, read higher. This is the "high-dose-hook" effect. We recommend to dilute and re-assay a patient sample that does not agree with clinical information. For this ELISA kit, a PSA concentration between 50 µg/L and 10 000 µg/L will generally produce an assay response greater than 50 µg/L. Over this 10 000 µg/L, the assay response will be lower than 50 µg/L.
8. Serum PSA concentration should not be interpreted as absolute evidence for the presence or absence of malignant disease. Patients with confirmed prostatic carcinoma may have pretreatment serum PSA concentrations within the range of concentrations observed in healthy individuals. Additionally, elevated PSA concentrations may be observed in the sera obtained from patients with nonmalignant diseases of the prostate, especially benign prostatic hyperplasia and prostatis. The definitive proof of cancer of the prostate relies upon histopathology and analysis of the biopsy specimen.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to expiration date on the label.

	96 tests

1. **MICROTITER PLATE** (Part ESEL-1) ***96 wells***
   Pre-coated with polyclonal anti-PSA immobilized into the well.

2. **CONJUGATE** (Part ESEL-2) ***22 mL***
   Anti-PSA antibody conjugated to horseradish peroxidase (HRP) in a stabilizing solution, thimerosal 0.1%. Ready to use.

3. **STANDARD** - **50 µg/L** (Part ESEL-3) ***1 mL***
   Protein matrix of human PSA and 0.01% sodium azide as a preservative.

4. **STANDARD** - **25 µg/L** (Part ESEL-4) ***1 mL***
   Protein matrix of human PSA and 0.01% sodium azide as a preservative.

5. **STANDARD** - **10 µg/L** (Part ESEL-5) ***1 mL***
   Protein matrix of human PSA and 0.01% sodium azide as a preservative.

6. **STANDARD** - **2 µg/L** (Part ESEL-6) ***1 mL***
   Protein matrix of human PSA and 0.01% sodium azide as a preservative.

7. **STANDARD - 0.2 µg/L** (Part ESEL-7) ***1 mL***
   Protein matrix of human PSA and 0.01% sodium azide as a preservative.

8. **STANDARD** - **0 µg/L** (Part ESEL-8) ***5 mL***
   Protein matrix of buffered protein base with 0.01% sodium azide as a preservative.

9. **SUBSTRATE – 2 VIALS** (Part ESEL-9) ***11 mL***
   Buffered solution with TMB.

10. **ASSAY BUFFER** (Part ESEL-10) ***54 mL***
    Buffered protein matrix with 0.2% NMS and 0.01% sodium azide as preservative.

12. **WASH BUFFER** (Part ESEL-11) ***100 mL***
    Concentrated solution of saline phosphate buffer with thimerosal as a preservative. Complete the content of the bottle to one (1) liter with deionized or distilled water.

13. **STOP SOLUTION** (Part ESEL-12) ***11 mL***
    2 M Sulfuric Acid (H_2SO_4). **CAUTION: Caustic Material!**

WARNING: Sodium azide may react with lead and copper to form explosive azides. Flush with copious quantities of water.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision pipettes (20 µL) with disposable tips or SMI pipette.
2. 8 channels pipette (50 and 200 µL) with disposable tips
3. Plate shaker set at 100 ± 10 rpm
4. Microplate reader with filter at 414 nm or 405 nm
5. Multichannel pipette with a repeater
6. Deionized or distilled water
7. Absorbent paper

PRECAUTIONS

1. All materials in this kit may be used only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material to humans or animals.
2. Respect laboratory quality control rules.
3. Reagents are matched in each kit and therefore, reagents from different lot numbers should not be mixed.
4. This should not be used after the expiration date.
5. Optimal results will be obtained by strict adherence to this protocol.
6. The kit contains sodium azide and thimerosal as preservatives. These are toxic and therefore, all reagents should be handled carefully to avoid ingestion or skin contact.
7. The stopping solution contains sulfuric acid. This solution should be handle with caution, avoiding contact with skin.
8. Prior to assay, warm all reagents to ambient temperature by allowing them to stand at room temperature (22 ± 2°C). Gently mix all reagents.

SAMPLE PREPARATION

No special preparation of the patient is necessary, but no massage of the prostate, no rectal examination, no biopsy and no prostatectomy within 48 hours before collection of the blood specimen must be done. Otherwise the PSA level may be elevated.

Serum is required for the ELISA-PSA. A whole blood specimen should be taken by standard medical technique. Allow the blood to clot and separate the serum by centrifugation.

The serum sample should be assayed within 24 hours after collection if the specimen is stored at 2-8°C. If the assay can not be performed within 24 hours, the serum should be frozen at –20°C for up to 1 year. Frequent freezing and thawing should be avoided.

Turbid sera or samples containing particulate matter should be centrifuged prior to assay. Specimen containing greater than 50 µg/L of PSA should be diluted with the 0 standard solution and re-assayed.

PREPARATION OF REAGENTS

Washing solution: Dilute the 100 mL solution to 1 liter with deionized water or distilled water. All other reagents supplied are ready to use.

ASSAY PROCEDURE

- DO NOT INTERCHANGE REAGENTS BETWEEN KITS BEARING DIFFERENT LOT NUMBERS.
- ALL REAGENTS AND PATIENT SAMPLES SHOULD BE BROUGHT TO ROOM TEMPERATURE (22 ± 2°C) BEFORE ASSAYING.
- ALL REAGENTS AND PATIENT SAMPLES SHOULD BE MIXED BY SWIRLING OR GENTLY VORTEXING. DO NOT INDUCE FOAMING.

Refer to the assay procedure, Table I. Tests should be run preferably in duplicate.

1. Firstly, Pipette 200 µL of Assay Buffer into each well. Secondly, Pipette 20 µL of standard, control or patient sample into the corresponding wells.
2. Incubate for sixty (60) minutes on the plate shaker (100 ± 10 rpm) at room temperature (22 ± 2°C).
3. Wash manually, precautions must be taken to avoid cross-contamination between wells. Decant the well content by inverting the plate over a container and without re-inverting, blot the plate against absorbing paper. Wash each well three times with 300 µL of washing solution. At the last wash, decant completely the washing solution by tapping the plate against absorbing paper until no trace of water is visible on the paper.
4. Pipette 200 µL of anti-PSA antibody conjugated with HRP in to each well.
5. Incubate for sixty (60) minutes on the plate shaker (100 ± 10 rpm) at room temperature (22 ± 2°C).
6. Decant and wash the wells three times with 300 µL of washing solution as in step 3.
7. At timed intervals, pipette 200 µL of the enzyme substrate TMB solution to each well.
8. Incubate for 15 minutes on the plate shaker (100 ± 10 rpm) at room temperature (22 ± 2°C).
9. Add 50 µL of a stopping solution and shake the microplate for homogenizing.
10. Measure the absorbance at 414 nm or 405 nm using a microplate reader.

NOTE: READ THE ABSORBANCES IMMEDIATELY AFTER COMPLETING THE ASSAY.

TABLE I
ELISA-PSA COATED WELL PROCEDURE

Wells	Identification	Assay Buffer	Assay Volume			Conjugate			Substrate		Stop Solution	
A_1A_2	0 µg/L											
B_1B_2	0.2 µg/L											
C_1C_2	2 µg/L											
D_1D_2	10 µg/L	200 µL	20 µL	INCUBATION	DECANT & WASH	200 µL	INCUBATION	DECANT & WASH	200 µL	INCUBATION	50 µL	READ AT 414 nm
E_1E_2	25 µg/L											
F_1F_2	50 µg/L											
G_1G_2	Control											
H_1H_2	Unknown											
etc…	etc…			1	2		1	2		3		

1. Incubate 60 minutes on the plate shaker (100 ± 10 rpm) at room temperature (22 ± 2°C)
2. Wash 3 times with a multichannel pipette (refer to the washing procedure)
3. Incubate 15 minutes on the plate shaker (100 ± 10 rpm) at room temperature (22 ± 2°C)

CALCULATION OF RESULTS

DO NOT SUBSTITUTE ANY PART OF THESE SAMPLE DATA FOR YOUR OWN.

Refer to the sample data and calculations, Table II and figure 1.

- The absorbance value of the standard zero should not be exceeding 0.120 however, it is an indication of careless washing and the assay must be repeated.
- Examine data for acceptance consistency with quality control guidelines. Aberrant values should be rejected.
- For each standard, control and unknown sample, the optical density values are averaged (if there is duplicate). Subtract the mean of the absorbance values of the zero standard from mean absorbance values of other standards, controls and samples.
- On millimeter paper using the ordinate for the optical density and the abscissa for the standard concentrations (µg/L), a smooth standard curve is plotted. The values of the control and unknown samples are read directly from the standard curve.

TYPICAL DATA

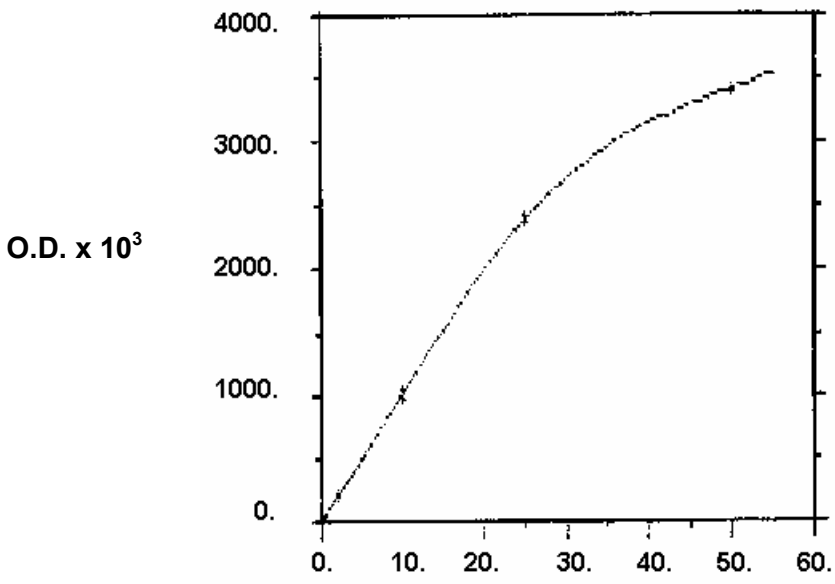
EXAMPLE
Results of a typical standard run are shown below:

TABLE II

WELLS	OPTICAL DENSITY at 414 nm	CONCENTRATION (µg/L)
0 µg/L	0.000	--
0.2 µg/L	0.022	--
2 µg/L	0.195	--
10 µg/L	1.007	--
25 µg/L	2.402	--
50 µg/L	3.422	--
Control	0.127	1.3
Unknown	0.357	3.6
Unknown	2.788	31.3

FIGURE 1

EXAMPLE OF PSA STANDARD CURVE
(Plotted from data on Table II)



PERFORMANCE CHARACTERISTICS

1. **SPECIFICITY:** The antibodies used in the ELISA-PSA kit are highly specific for PSA, with a relatively low cross-reactivity to other proteins and polypeptides, lipids or chemotherapeutic agents present in patient samples.

ANTIGENS	AMOUNT ADDED	CROSS-REACTIVITY
Proteins		
AFP	10 µg/mL	Not-detectable
CEA	10 µg/mL	Not-detectable
HCG	10 µg/mL	Not-detectable
Lactalbumin	10 µg/mL	Not-detectable
PAP	1 µg/mL	Not-detectable
Interfering Substances		
Bilirubin	20 mg/dL	Not-detectable
Triglyceride	1 500 mg/dL	Not-detectable
Chemotherapeutic Agents		
Cyclophosphamid	800 µg/mL	Not-detectable
Methotrexate	50 µg/mL	Not-detectable
Doxorubicin-HCL	20 µg/mL	Not-detectable
Diethystilbestrol	2 µg/mL	Not-detectable
Flutamide	10 µg/mL	Not-detectable
Hemolysis		
Red blood cell	10 µg/mL	Not-detectable

2. **PRECISION & REPRODUCIBILITY:** The precision of the method was evaluated by assaying human sera having different concentrations of PSA.

a) **Intra-Assay Variation:**

Parameters	Samples		
	1	2	3
Number of duplicates (N)	20	20	20
Mean (µg/L)	0.99	3.29	21
Standard deviation (µg/L)	0.08	0.17	0.83
Coefficient variation (%)	7.8	5.3	2.6

b) **Inter-Assay Variation:**

Parameters	Samples		
	1	2	3
Number of duplicates (N)	20	20	20
Mean (µg/L)	1.2	4.30	30.43
Standard deviation (µg/L)	0.10	0.25	1.08
Coefficient variation (%)	8.4	5.8	3.5

3. **SENSITIVITY:** the minimum detectable concentration or sensitivity is defined as that concentration of PSA that corresponds to the cpm that are two standard deviations greater than the mean cpm of 20 replicate determinations of the Zero standard. The sensitivity is estimated to be less than 0.15 µg/L.

4. **ACCURACY:** or dilution study; two (2) serums were diluted and run in the ANOGEN PSA-ELISA kit. The results are as follows:

Samples	Expected value (µg/L)	Observed value (µg/L)	Recovery (%)
1	6.30	6.34	100
2	4.67	4.56	98
3	10.10	10.91	108

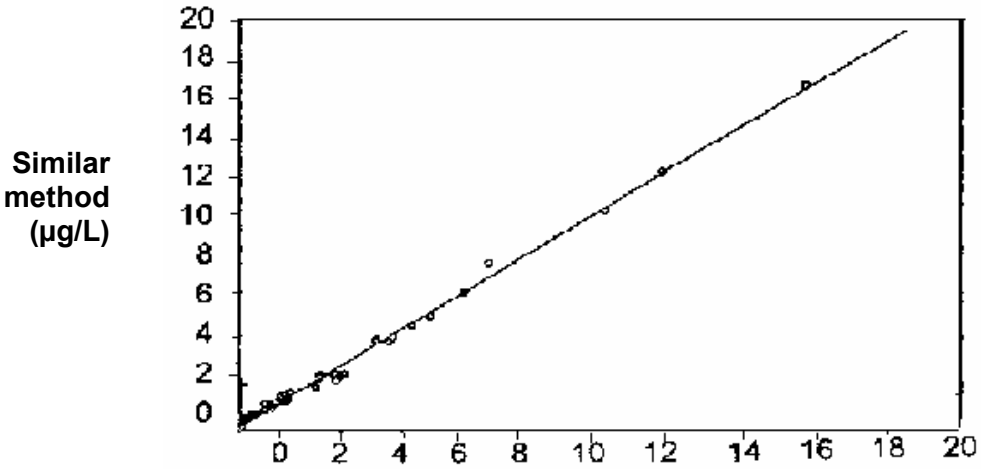
5. **LINEARITY:** Or dilution study; two (2) serums were diluted and run in the ANOGEN PSA-ELISA kit. The results are as follows:

Samples	Dilution Factor	Theoretical value (µg/L)	Experimental value (µg/L)
1	1/10	18.56	18.56
	1/20	9.28	9.67
	1/40	4.64	5.78
	1/80	2.32	2.54
2	1/10	19.58	19.58
	1/20	9.79	10.79
	1/40	4.90	5.25
	1/80	2.45	2.56

6. **CORRELATION STUDY:** Clinical samples were analyzed by the ANOGEN PSA-ELISA kit in parallel with a similar method. The results of this study are as follows :

N	=	48
Intercept	=	-0.04
Slope	=	1.04
Correlation coefficient	=	0.998

REGRESSION LINE FROM CORRELATION STUDY



7. **EXPECTED NORMAL VALUES:** Healthy men have a PSA concentration of 4.0 µg/L or less.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to check the assay performance. Commercial controls are suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

REFERENCES

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2. P.Lange et al., The value of serum prostatic-specific antigendeterminations before and after radical prostactectomy. *J. Urol*. 141,873 (1989).
3. T. Stamey et al., Prostate-specific antigen as a serum marker for adenocarcinima of the prostate. *N. Engl. J. Med*. 317, 909-916 (1987).