BIOGENIX
INC. PVT. LTD.

Total PSA

Enzyme Immunoassay for Quantitative Determination of Total Prostate-Specific Antigen in Human Serum

1. SYMBOL LEGEND

IVD	In vitro diagnostic medical device	RCNS	Reconstitute with specified volume of liquid
REF	Catalogue number	LOT	Batch code
	Use by		Manufacturer
	Date of manufacture		Consult operating instructions
	Temperature limitation		Biological risks
	Caution, consult accompanying documents	CONJ	Conjugate
MP	Coated microplate (96 wells)	SUB	Substrate
WASH P 20X	Wash solution, 20X concentrated	STOP	Stop solution
CAL	Calibrators	DIL	Sample diluent
CONTROL	Controls	OD	Optical density
	Contains sufficient for <n> tests	H2O	Deionized or distilled water
 C	Corrosive	TRIAL 5000X	Trial
		 Xi	Irritant

2. INTENDED USE

Total PSA kit is intended for the **quantitative determination of prostate-specific antigen (PSA) in human serum.**

PSA is a single-chain glycoprotein with a molecular mass of about 32 000 Da. It is a serine protease that is produced only by human prostate epithelium. Normally PSA is secreted into seminal fluid in high concentrations. There it exhibits its enzymatic activity and is directly involved into liquefaction of seminal clot. In serum PSA persists in low concentrations. Increase of PSA concentration in serum is a sign of prostate pathologies, such as benign hyperplasia or malignant degeneration of prostate tissues. PSA determination is widely used for the revealing and monitoring of prostate cancer patients.

It was demonstrated that PSA makes stable complexes with different protease inhibitors. The main part of the serum PSA is a complex with α_1-antichymothrypsine (PSA-ACT). But there is a great difference in free PSA/PSA-ACT ratio in different groups of patients. In the case of benign hyperplasia the part of free PSA is higher than in the case of prostate cancer.

3. PRINCIPLE OF TEST

Total PSA kit is a “sandwich” type of solid-phase enzyme immunoassay, based on two monoclonal antibodies that are specific for different epitopes of PSA molecule. One of these antibodies is conjugated with horseradish peroxidase (HRP); the other is coated onto the inner surface of microwells. PSA molecules from the serum sample bound to both immobilized antibody and anti-PSA-peroxidase conjugate (Fig. 1). Then the wells are washed with wash solution to remove any material not

bound to the inner surface of the wells. Quantity of the bound conjugate is directly proportional to the PSA level in sample.

During incubation with substrate the colour is developing. The intensity of the colour is directly proportional to the concentration of PSA in specimens or calibrators. PSA concentration in the patient sample is read from a standard curve that is processed in each assay.

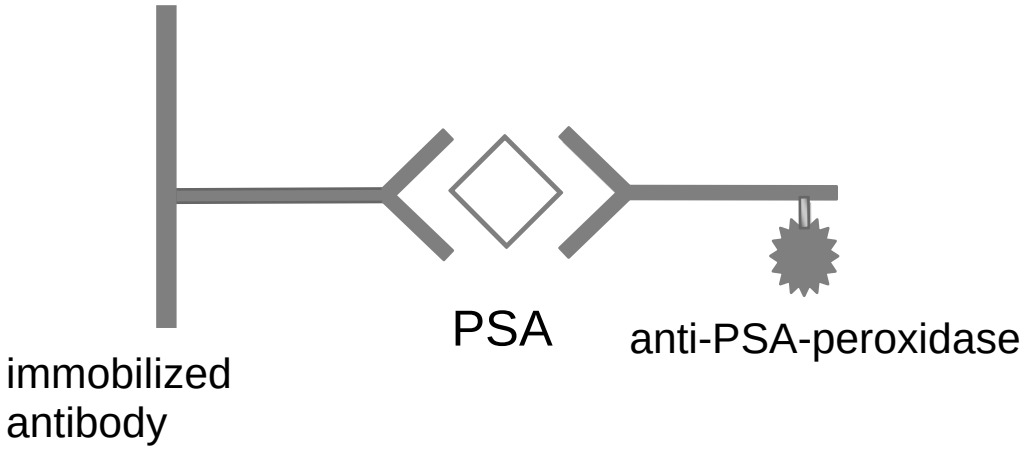

Fig. 1. Assay scheme

4. STORAGE AND STABILITY OF THE KIT

The expiration date of the kit is printed on the box label; the expiration date for each component is printed on the respective label.

Total PSA kit should be stored at 2-8 °C upon receipt, preferably in the original kit box, until the expiration date. Storage at +25 °C is allowed but for no more than 5 days.

Shelf life of the kit is 18 months.

After initial opening the kit is stable for 1 month if stored at 2-8 °C.

If used in several separate experiments, kit contents should be stored as follows:

- unused strips: in a firmly closed resealable zipper bag at 2-8 °C until the expiration date;
- opened vials with calibrators and control (ready-to-use or reconstituted), conjugate, sample diluent: at 2-8 °C for no more than 1 month after opening;
- opened vial with substrate: at 2-8 °C for no more than 1 month after opening, protected from light;
- opened vials with concentrated Trial, concentrated wash solution and stop solution: at 2-8 °C until the expiration date;
- wash solution and Trial solution prepared for use: at room temperature (18-25 °C) for no more than 5 days, in a firmly closed bottle.

5. SAMPLE COLLECTION AND STORAGE

Collect blood by venipuncture. After clotting, the serum is separated by centrifugation.

Do not use plasma, haemolyzed (bright red) or lipemic (milky) serum samples as well as samples containing sodium azide as preservative.

Store serum samples at 2-8 °C for no more than 2 days. Aliquot and freeze samples for longer storage (-20 °C and lower). Avoid repeated freezing.

6. EXPECTED VALUES

PSA concentrations up to 4 ng/mL was classified as normal. PSA concentrations between 4 ng/mL and 10 ng/mL are general for benign urological diseases, the higher values are specific for the prostate malignancies. These limits should be considered as guidelines only.

It is highly recommended for each laboratory to determine its own reference range of PSA concentrations.

7. QUALITY CONTROL

It is recommended to use control samples according to the state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

Allow all the reagents to reach room temperature, and then thoroughly stir.

8. REAGENT PREPARATION

MP Keep **microplate** at room temperature (18-25 °C) for at least 30 minutes before opening the bag. Place required number of strips into strip holder. Place unused strips onto the resealable zipper bag and reseal duly.

CAL **CONTROL** **Calibrators and Control**

Liquid calibrators and control are ready to use.

Prepare lyophilized calibrators and control as follows. Gently tap on the vial caps to knock off all the dry matter. Open the vials and carefully place the caps upside down on the clean dry surface. Add 0.5 mL of distilled or deionized water to each vial with lyophilized calibrators and control, close vials with the corresponding caps and leave for 10 min at room temperature without stirring. Then stir gently avoiding foaming, until the dry matter is completely dissolved. Leave for another 10 minutes at room temperature stirring gently periodically. Make sure that no dry matter is left on the caps and walls of the vials.

WASH **P** Prepare required volume of **wash solution** by dilution of the concentrate 20-fold with distilled or deionized water. For example:

5 mL of **WASH P** **20X** + 95 mL of water.

Mix thoroughly, avoid foaming.

SUB Protect **substrate** from direct light.

9. SAMPLE PREPARATION

Allow samples to reach room temperature. Stir samples gently in order to ensure homogeneity.

If expected PSA concentration in the samples is higher than in calibrator 5, the samples should be diluted 5-fold (Sample 1) and 25-fold (Sample 2) with the sample diluent **DIL** as follows:

Sample 1 (5-fold dilution):

120 µL of sample diluent DIL + 30 µL of serum sample, mix thoroughly (for manual test).

400 µL of sample diluent DIL + 100 µL of serum sample, mix thoroughly,

Sample 2 (25-fold dilution):

120 µL of sample diluent DIL + 30 µL of Sample 1, mix thoroughly (for manual test).

480 µL of sample diluent DIL + 120 µL of Sample 1, mix thoroughly,

If PSA concentration in the Sample 2 is higher than in calibrator 5, the samples should be diluted 50-fold (Sample 3) and 100-fold (Sample 4) with the sample diluent DIL as follows:

Sample 3 (50-fold dilution):

50 µL of sample diluent DIL + 50 µL of Sample 2, mix thoroughly (for manual test).

250 µL of sample diluent DIL + 250 µL of Sample 2, mix thoroughly,

Sample 4 (100-fold dilution):

50 µL of sample diluent DIL + 50 µL of Sample 3, mix thoroughly (for manual test).

150 µL of sample diluent DIL + 150 µL of Sample 3, mix thoroughly,

Do not store diluted samples.

10. PERFORMANCE CHARACTERISTICS OF THE ASSAY

10.1. Calibration- Traceability

Total PSA kit was calibrated against the Stanford Reference Standard (90% PSA-ACT + 10% free PSA).

10.2. Linearity test (dilution test) for manual kit

Serial dilutions of four human serum samples with predetermined total PSA concentration in calibrator 0 were assayed with **Total PSA kit** with the following result:

Sample	Dilution	Measured concentration, ng/mL	Expected concentration, ng/mL	Measured/expected concentration ratio, %
1	Undiluted	25.3		
	1:2	13.3	12.7	104.7
	1:4	7.0	6.8	102.9
	1:8	3.7	3.4	108.8
	1:16	1.7	1.7	100.0
	1:32	0.9	0.9	100.0
2	Undiluted	18.9		
	1:2	8.4	9.3	90.3
	1:4	4.5	4.7	95.7
	1:8	2.2	2.4	91.7
	1:16	1.0	1.1	90.9
3	Undiluted	29.8		
	1:2	13.5	14.9	90.6
	1:4	7.1	7.7	92.2
	1:8	3.9	3.7	105.4
	1:16	2.0	1.9	105.2

10.2. Analytical Sensitivity

Analytical sensitivity of **Total PSA kit**, or the lowest detectable concentration that can be distinguished from zero calibrator, is 0.2 ng/mL. It was defined as mean OD of 10 replicates of calibrator 0 plus 2SD.

10.4. Specificity

Both monoclonal antibodies used in the kit show an equimolar interaction with PSA as well with PSA-ACT complex.

10.5. Measurement Range

Total PSA kit was validated for measurement of PSA concentration within the concentration diapason (without dilution) of 0.2 - 30 ng/mL.

10.6. Hook Effect

For **Total PSA** kit **high dose hook effect** was not detected for concentrations up to 1200 ng/mL. **High dose hook effect** was determined by spiking calibrator 0 with antigen.

10.7. Precision (intra- and inter-assay CV)

For **intra-assay CV** determination 8 serum samples were run, each in 9 replicates. The results are shown below.

Sample	Mean total PSA concentration, ng/mL	Intra-assay CV	
		SD	CV, %
HS 1	0.3	0.02	6.2
HS 2	0.6	0.03	5.7
HS 3	1.4	0.05	3.3
HS 4	1.9	0.09	4.8
HS 5	2.5	0.09	3.7
HS 6	2.9	0.18	6.3
HS 7	3.9	0.27	6.9
HS 8	4.8	0.35	7.2

For **inter-assay CV** determination, 8 serum samples were assayed 9 times by different operators with 1-week interval. Each specimen was run in 2 replicates. The results are shown below.

Sample	Mean total PSA concentration, ng/mL			Inter-assay precision	
	1 assay	2 assay	3 assay	SD	CV, %
HS 1	0.31	0.27	0.33	0.050	10.1
HS 2	0.63	0.69	0.58	0.055	8.7
HS 3	1.44	1.24	1.40	0.106	7.8
HS 4	1.89	1.95	2.03	0.070	3.6
HS 5	2.48	2.59	2.37	0.110	4.4
HS 6	2.86	2.71	2.73	0.081	2.9
HS 7	3.87	3.98	3.56	0.218	5.7
HS 8	4.84	5.03	5.12	0.143	2.9

11. LIMITATION OF THE METHOD

Any clinical diagnosis should not be based on the results of in vitro diagnostic method alone. For diagnosis establishment, a physician is supposed to consider all available clinical and laboratory findings. The highest measurable PSA concentration without dilution is about 30 ng/mL.

12. SAFETY PRECAUTIONS

- **This kit is for in vitro diagnostic use only.** Operator should follow the manual closely in order to ensure reliable data. The manual is valid for the present kit only, within the listed composition. Any substitution of kit components is not allowed by CE regulations.
- Do not use kit or its components after the expiration date indicated on the label. Take into account stability period for reconstituted reagents.

- Do not mix or use together reagents from different lots of the kit except substrate, stop solution and wash solution.
- Do not use substrate, stop solution and wash solution supplied by other vendors.
- Note that stop solution is 1N HCl solution. Avoid contacts with skin and mucosa. In case of contact rinse affected area thoroughly with plenty of water and seek medical advice.
- Take into account the following common procedural notes:
  - always pipette reagents into wells immediately after washing procedure;
  - avoid contamination of the solutions;
  - in case of partial use of the kit, dispense only required volume of the reagent into the tray;
  - do not pour unused reagents back into the original vials;
  - avoid exposure to direct sunlight during incubations;
  - always pipette reagents in the same order to minimize reaction time differences between wells; the total dispensing time for the calibrators, control and samples must not exceed 15 min;
  - the incubation temperature for all the immunological reactions must be kept at 37°C;
  - do not touch the bottom of the wells;
  - calibrators should be measured in each separate assay. It is also recommended to measure each time PSA concentration in the control.
- TMB solution should be colourless. Light colouring of solution is admissible. Avoid direct exposure of substrate to sunlight.

- Source materials of human origin used for kit components preparation were tested and found negative for HBsAg, anti-HIV and anti-HCV antibodies. However, none of known laboratory test guarantees absence of these viral agents. Therefore, all kit components and patient's samples should be handled as potentially hazardous.
- After usage strips, calibrators, controls, specimens and all consumables which contacted with specimens during handling, storage or assay (tubes, vials, gloves, pipette tips etc.) should be collected separately and sterilized by autoclaving. Instead of autoclaving pipette tips may be sterilized by disinfectant treatment. After sterilization all components and expendable materials may be utilized as non-dangerous garbage. Other components of the kit should be discarded into conventional garbage.
- During manual washing procedure do not discard the contents of the wells directly to drainage. Use a container with disinfectant solution.
- As the kit contains potentially hazardous material, the following precautions should be taken:
    - do not smoke, eat or drink while performing the assay;
    - always use protective gloves;
    - never pipette material by mouth;
    - in case of spilling, wipe up the spills promptly and wash affected area thoroughly using decontaminant.
- GLP including all general and individual regulations should be applied for the kit usage.

13. MATERIALS PROVIDED

13.1. Material Provided

MP	**Microplate:** 12 breakable 8-well strips (total 96 wells) coated with anti-PSA monoclonal antibodies	1 pcs
CONJ	**Conjugate:** solution contains anti-PSA monoclonal antibodies conjugated with HRP	14 mL, ready to use
0–5 CAL	**PSA calibrators:** protein-based solution or lyophilized reparations containing known PSA concentrations - 0; 1; 2.5; 5; 10; 30 ng/mL. For exact PSA concentrations, see vial labels	6 vials, 0.5 mL each; ready to use or lyophilized preparations
CONTROL	**PSA control:** protein-based solution or lyophilized preparation containing known PSA concentration. For exact range of PSA concentration, see vial labels 	0.5 mL, ready to use or lyophilized preparation
WASH P 20X	**Wash solution P, 20X concentrated:** surfactant in buffered saline,sufficient for preparation of 280 mL of solution	2x14 mL, concentrated
SUB	**Substrate (TMB solution):** 3,3',5,5'-tetramethylbenzidine solution in citrate buffer containing hydrogen peroxide	14 mL, ready to use
STOP	**Stop solution:** 1N HCl solution	14 mL, ready to use
DIL	**Sample diluent:** protein-based solution	3.0 mL, ready to use

13.2. Equipment and Materials Required but not provided

- 1-channel calibrated variable precision pipettes, with disposable tips;
- 8-channel calibrated variable precision pipette, with disposable tips;
- microplate incubator/shaker (37°C, shaking speed 400–800 rpm);
- manual or automatic equipment for rinsing wells;
- calibrated microplate reader (450 nm);
- vortex tube mixer;
- deionized or distilled water;
- graduated beaker and cylinder of appropriate volume;
- latex or plastic gloves;
- trays for pipetting reagents with 8-channel pipette;
- disinfectant;
- absorbent material (for manual wash).

13.3. Test procedure

Total PSA kit is designed for 96 tests. This is sufficient for 40 unknowns, 6 calibrators, 1 control and 1 blank (OD of TMB solution) in duplicates, provided that all the strips are used simultaneously.

13.3.1. Assay Procedure

(See assay scheme, section 13.5.)

A. Pipette**:**

- **100 µL** of **conjugate** **CONJ** into each well,

Leave wells A1-A2 empty for blank!

B. Pipette**:**

- **20 µL** of calibrators **CAL**, control **CONTROL** and patient's samples in duplicates**;**

except wells A1-A2.

C. Incubate for **1 hour at 37°C**

D. Wash 5 times, as described in section 13.3.2.

E. Pipette **100 µL** of **substrate SUB** into each well (including blank); incubate strips **at room temperature** (18-25 °C) **in the dark for 15-30 minutes**, depending on the colour intensity, or **10 minutes while shaking (500-800 rpm) at 37 °C**.

F. Pipette **100 µL** of **stop solution STOP** to all the wells in the same sequence and at the same speed as used for dispensing TMB substrate. Shake for 1–2 min at room temperature.

G. Read OD at **450 nm within 20 min.**

13.3.2. Wash Procedure

It is advisable to use an automatic microplate washer set at 5 wash cycles and a volume of 300 µL of wash solution per well per cycle.

If an automatic washer is not available, the wash procedure can be carried out manually as follows:

- remove the contents of the wells into a container with disinfectant;
- dispense 300 µL of wash solution, prepared according to section 8, into each well, shake the plate carefully for 5–10 sec and remove the contents of the wells; repeat 5 times;
- strike the wells sharply on absorbent material to remove any liquid residue.

13.4. Data Processing

If the reader cannot be adjusted to zero using the substrate blank in wells A1-A2, subtract mean OD value of wells A1-A2 from all OD values before further calculations.

Example:

OD (Cal 5) measured = 2.28 and OD (blank) = 0.06;

OD (Cal 5) calculated = 2.28 – 0.06 = 2.22

13.4.1. Data Reliability (for OD Measured at 450 nm)

The data should meet the following criteria:

- average blank OD (in wells A1-A2) ≤0.100;
- average OD of Cal 5 ≥1.5 (after blank subtraction);
- control's concentration must fall within the acceptability range that are shown on the vial labels.

If the data obtained do not meet the criteria, the results are considered unreliable and the test should be repeated.

13.4.2. Quantitative Determination

Specialized software for quantitative determination is recommended. Plot mean OD values of the calibrators at 450 nm versus their respective PSA concentrations using 4PL or 5PL fit (see typical standard curve, fig. 2). Calculate concentration of PSA in samples using standard curve.

Any extrapolation of the standard curve to PSA concentration above the nominal value of calibrator 5 is forbidden. In this case the sample should be diluted.

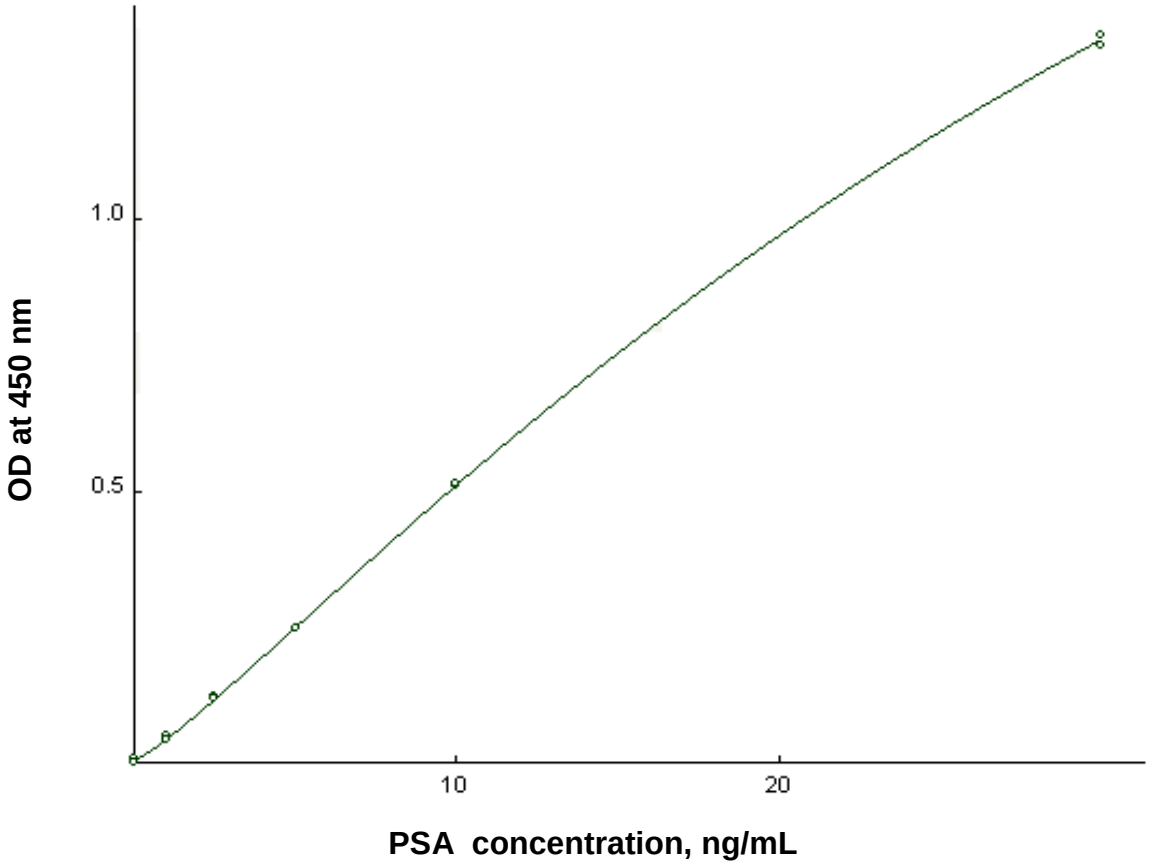

Fig. 2. Example of typical standard curve.
Do not use for evaluation of real assay data!

13.5. Assay scheme

Wells / Reagents	«Blank»	**CAL** **CONTROL**	Samples
CONJ	–	100 µL	100 µL
CAL **CONTROL**	–	20 µL	–
Samples	–	–	20 µL
Incubation No.1	60 min, 37 °C Incubator		
WASH **P** (diluted)	5 x 300 µL		
SUB	100 µL	100 µL	100 µL
Incubation No.2	15–30 min, 18-25 °C, in the dark		
	10 min, 37 °C Incubator		
STOP	100 µL	100 µL	100 µL
Stirring	1–2 min, 18-25 °C		
OD measuring	450 nm		
Calculations	Corresponding software		

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