FPSA Free Prostate-specific Antigen (Elisa)

Please note
The measured fPSA value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the fPSA assay method used. fPSA values determined on patient samples by different testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations. If there is a change in the fPSA assay procedure used while monitoring therapy, then the fPSA values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Intended use
The free PSA assay is an enzyme-linked immunosorbent assay (ELISA) for the in vitro quantitative determination of free prostate-specific antigen (fPSA) in human serum

Summary
PSA is a 32 kDa single chain glycoprotein serine protease with a chymotrypsin like speci city produced by the secretory epithelium of the prostate gland.1 PSA is normally secreted into the seminal uid and plays a functional role in the cleavage of the seminal vesicle proteins and the liquefaction of the seminal coagulum.2 Only low levels of PSA are normally present in the blood stream, and increasing serum concentrations indicate prostatic pathology, including benign prostatic hyperplasia and cancer of the prostate. Determination of PSA is now widely used for detection and management of patients with prostatic cancer and considered as the superior serological marker for cancer of the prostate.3
PSA has been shown to form stable complexes with different antiproteases and the dominating portion of PSA in patient serum occurs in complex with α1-antichymotrypsin
(PSA-ACT).4 However there are large variations in the relation between Free PSA and PSA- ACT complex between different individuals. A number of studies have found that the proportion of Free PSA is higher in benign prostatic disease as compared to prostatic cancer.

Test principle
Sandwich principle. Total duration of assay: 80 minutes.
• Sample, PSA-specific antibody coated microwells and enzyme labeled Anti-Free PSA
monoclonal antibody are combined.
• During the incubation, Free PSA presents in the sample is allowed to react simultaneously with the two antibodies, resulting in the Free PSA molecules being sandwiched between the solid phase and enzyme-linked antibodies.
• After washing, a complex is generated between the solid phase, the Free PSA within the sample and enzyme-linked antibodies by immunological reactions.
• Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
• The absorbance is proportional to the amount of Free PSA in the sample.

Reagents
Materials provided
• **Coated Microplate - symbol** fPSA PLATE 8 x 12 strips, 96 wells, pre-coated with mouse monoclonal PSA-specific antibody.
• **Calibrators - symbol** fPSA PLATE 6 vials, 1 mL each, ready to use; Concentrations:
0(A), 1(B), 2.5(C), 5(D), 10(E) and 20(F) ng/mL.
• **Enzyme Conjugate – symbol** fPSA CONJ 1 vial, 11 mL of HRP (horseradish peroxidase) labeled Anti-Free PSA monoclonal antibody in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin300 preservative.
• **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
• **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
• **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
• **IFU** - 1 copy.
• **Plate Lid**: 1 piece.

Materials required (but not provided)
• Microplate reader with 450nm and 620nm wavelength absorbent capability.
• Microplate washer.
• Incubator.
• Plate shaker.
• Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
• Absorbent paper.
• Distilled water

Precautions and warnings
• For in vitro diagnostic use only.
• Exercise the normal precautions required for handling all laboratory reagents.
• Disposal of all waste material should be in accordance with local guidelines.
• Do not use reagents beyond the labeled expiry date.
• Do not mix or use components from kits with different batch codes.
• All the specimen and reaction wastes should be considered potentially biohazard. The
handling of specimens and reaction wastes should be in accordance with the local
regulations and guidelines.
• The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
• Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
• Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
• Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
• For information on hazardous substances included in the kit please refer to the Materials
Safety Data Sheet (MSDS), which is available on request.
• Do not smoke, drink, eat or apply cosmetics in the work area.
• Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face
protection when handling samples and reagents. Wash hands after use.
• If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.

Storage and stability
• Store at 2-8°C.
• Seal and return unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
• Human serum is recommended for this assay.
• Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 7 days at
2-8°C, and 1 month at -20°C. Freeze only once.
• Do not use heat-inactivated samples.
• Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
• Avoid grossly hemolytic, lipemic or turbid samples.

Quality control
Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate.
Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure
Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25
°C) before measurement. Mix all reagents through gently inverting prior to use.
• Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.
• Add 25 µL of calibrators or samples to each well.
• Add 100 µL of enzyme conjugate to each well.
• Shake the microplate gently for 30 seconds to mix.
• Cover the plate with a plate lid and incubate at 37 °C for 60 minutes.
• Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and
blot the plate dry with absorbent paper.
• Add 350 µL of wash solution, decant (tap and blot) or aspirate. Repeat 4 additional times for a total of 5 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
• Add 100 µL of substrate to each well.
• Cover and incubate at ambient temperature (18-25°C） in the dark for reaction for 20
minutes. Do not shake the plate after substate addition.

• Add 50 µL of stop solution to each well.
• Shake for 15-20 seconds to mix the liquid within the wells. It is important to ensure that
the blue color changes to yellow completely.
• Read the absorbance of each well at 450 nm (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be
read within 30 minutes of adding the stop solution.
Calculation
• Record the absorbance obtained from the printout of the microplate reader.
• Calculate the mean absorbance of any duplicate measurements and use the mean for
the following calculation.
• Plot the common logarithm of absorbance against concentration in ng/mL for each calibrator.
• Draw the best-fit curve through the plotted points on linear graph paper. Point-to-Point method is suggested to generate a calibration curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Sample	Value (ng/mL)	Absorbance
Calibrator A	0	0.014
Calibrator B	1	0.180
Calibrator C	2.5	0.479
Calibrator D	5	1.023
Calibrator E	10	2.015
Calibrator F	20	2.555
Control 1	1.75	0.330
Control 2	8.16	1.650
Sample	1.58	0.295

Limitations - interference
• The assay is unaffected by icterus (bilirubin < 1000 µmol/L or < 58 mg/dL), hemolysis (Hb< 1.0 mmol/L or < 1.61 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 200 nmol/L or < 49 ng/mL).
• No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.
• There is no high-dose hook effect at Free PSA concentrations up to 5000 ng/mL.
• In vitro tests were performed on 28 commonly used pharmaceuticals. No interference with the assay was found.
• The level of Free PSA alone should not be used as evidence for the presence or absence of malignant disease. The results of the test should be interpreted only in conjunction with other investigations and procedures in the diagnosis of disease and the management of patients. The Free PSA test should not replace any established clinical examination.
• The calibrators of the Free PSA ELISA kit should not be used for recovery studies of Free PSA. For recovery studies it is recommended to use a highly elevated patient sample.
• Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti- mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
• For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range
0.01-20 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.01 ng/mL. Values above the measuring range are reported as > 40 ng/mL (or up to 200 ng/mL for 20-fold diluted samples).

Lower detection limit
0.01 ng/mL
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 18).

Limit of Blank (LoB) and Limit of Detection (LoD)
LoB = 0.006 ng/mL LoD = 0.008 ng/mL
Both Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.
The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free
samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.
The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95
%).

Expected values
Free PSA measurements may be used in conjunction with an equimolar test such as tPSA ELISA (BL167733) for total PSA in order to generate the ratio of Free PSA/Total PSA. Serum specimens from 45 men objectively diagnosed with benign prostate hyperplasia
(BPH) and 67 men diagnosed with prostate cancer (PCa) were analysed using tPSA ELISA
and Free PSA ELISA:

Diagnosis (n)	fPSA/tPSA			fPSA/tPSA	
	Median	Max.	Min.	Mean	95% confidence interval
BPH (54)	0.18	0.05	0.43	0.20	(0.17-0.23)
PCa(87)	0.09	0.02	0.55	0.11	(0.09-0.15)

The choice of a cut-off to be used in clinical practice depends upon the clinical application, i.e. whether optimised sensitivity or speci city is desired. Sensitivities (% PCa correctly detected) and Speci cities (% BPH correctly detected) for dif- ferent FPSA/TPSA ratio cut- offs are shown below:

fPSA/tPSA	Clinical specificity (BPH>cut-off)			Clinical specificity (PCa ≤ cut-off)		
cut-off	n	%	95% confidence interval	n	%	95% confidence interval)
0.25	16 (54)	29.6	(17-45)	80(87)	92.0	(84-96)
0.15	40 (54)	74.0	(55-86)	76(87)	87.3	(75-90)
0.09	50 (54)	92.6	(83-98)	39(87)	44.8	(30-52)

It is recommended that each laboratory investigate the transferability of the above expected values to its own patient population and assay performance.

Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision
Precision was determined using reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:

	Repeatability			Intermediate precision		
Sample	Mean ng/mL	SD ng/mL	CV %	Mean ng/mL	SD ng/mL	CV %
Human Serum 1	0.45	0.03	6.4	0.48	0.04	8.1
Human Serum 2	2.76	0.14	5.2	2.68	0.17	6.2
Human Serum 3	5.84	0.29	4.9	6.05	0.35	5.8
PC Universal 1	1.05	0.06	5.8	1.03	0.05	5.3
PC Universal 2	3.77	0.16	4.3	3.84	0.17	4.5

Method comparison
A comparison of the Free PSA assay (y) with the Roche Elecsys PSA free (x) using clinical samples gave the following correlations: Number of samples measured: 156
Linear regression
y = 0.9884x - 0.0197 r = 0.9822
The sample concentrations were between approx. 0 and 20 ng/mL.

Analytical specificity
The Free PSA ELISA is based on two mouse monoclonal antibodies, directed against two distinct epitopes exposed in Free PSA. This antibody combination provides an assay specific for Free PSA showing <1% cross-reactivity to the PSA-ACT complex.

Functional sensitivity
0.05 ng/mL
The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of 20 %.

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