abia PSA free

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IVD

CE 0483

Note: Changes highlighted ★

abia

Intended use

abia PSA free is an enzyme immunoassay for the quantitative determination of free prostate specific antigen (fPSA) concentration in human serum. For professional use only.

Clinical value

Human Prostate Specific Antigen (PSA) is a 33 kDa serine proteinase which, in human serum, is predominantly bound to alpha 1-antichymotrypsin (PSA-ACT) and alpha 2-macroglobulin (PSA-AMG).

Trace amounts of alpha 1-antitrypsin and inter-alpha trypsin inhibitor bound to PSA can also be found. Any remaining PSA is in the free form (fPSA). Current methods of screening men for ★ prostate cancer utilize the detection of the major PSA-ACT form. However, elevated serum PSA levels have also been attributed to benign prostatic hyperplasia and prostatitis, leading to a large percentage of false positive screening results.

A potential solution of this problem involves the determination of free PSA levels. Preliminary studies have suggested that the percentage of free PSA is lower in patients with prostate cancer than those with benign prostatic hyperplasia (BPH). Thus, the measurement of free serum PSA in conjunction with total PSA, can improve specificity of prostate cancer screening in selected men with elevated total serum PSA levels, which would subsequently reduce unnecessary prostate biopsies with minimal effects on cancer detection rates.

★ According to NCCN Guidelines, use of fPSA/tPSA ratio for the early detection of prostate cancer is recommended for the following individuals:

- patients of 45 – 75 years of average risk group, with total PSA level › 3 ng/ml and/or very suspicious digital rectal examination result
- patients of 40 – 75 years of high risk group, with total PSA level › 3 ng/ml and/or very suspicious digital rectal examination result
- healthy individuals over 75 years with total PSA level › 4 ng/ml and/or very suspicious digital rectal examination result
- ndividuals over 50 years with a non-suspicious digital rectal examination and PSA levels between 4 ng/ml and 10 ng/ml

It is strongly recommended to testing laboratories to take advantage of the regulations or guidelines of their country. fPSA/tPSA ratio should be used as a supplementary marker and an aid to a physician, but should never be used solely for decision making (see also Limitations of test).

Principle of the test

abia PSA free is a two-step immunoassay, based on the principle of the "sandwich" method. ★

The assay system utilizes high affinity and specificity monoclonal antibodies (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact fPSA molecule.

The test sample is allowed to react simultaneously with the two antibodies, resulting in the fPSA molecules being sandwiched between the solid phase and enzyme-linked antibodies.

The unbound components are removed by washing. After addition of the solution containing TMB and hydrogen peroxide, the wells with bound conjugate develop a blue colour which is converted to yellow after the reaction has been stopped with sulphuric acid.

The colour intensity is directly proportional to the concentration of fPSA in the specimen and can be read at 450 nm.

Kit contents

	S	
PSA Ab coated plate	1	polystyrene plate 12 × breakable 8-well strips coated with monoclonal anti-fPSA Ab
Conjugate	1 × 12 ml	ready to use; HRP-labeled monoclonal anti-PSA; transparent or slightly opalescent pink liquid
Sample diluent	1 × 6.0 ml	protein based buffer; transparent or slightly opalescent blue liquid
Calibrator 0	1 × 2.0 ml	protein based buffer not containing fPSA; pale yellow liquid
Calibrator 1	1 × 0.5 ml	protein based buffer containing fPSA in concentration approx. 0.50 ng/ml; pale yellow liquid
Calibrator 2	1 × 0.5 ml	protein based buffer containing fPSA in concentration approx. 1.00 ng/ml; pale yellow liquid
Calibrator 3	1 × 0.5 ml	protein based buffer containing fPSA in concentration approx. 2.00 ng/ml; pale yellow liquid
Calibrator 4	1 × 0.5 ml	protein based buffer containing fPSA in concentration approx. 5.00 ng/ml; pale yellow liquid
Calibrator 5	1 × 0.5 ml	protein based buffer containing fPSA in concentration approx. 10.00 ng/ml; pale yellow liquid
Control serum	1 × 0.5 ml	protein based control containing fPSA; pale yellow liquid ★
Washing solution (concentrated 25-fold)	1 × 50 ml	phosphate saline buffer; colourless or pale yellow liquid
TMB/substrate solution	1 × 12 ml	ready to use; citric acid buffer containing TMB and H_2O_2; colourless liquid
Stopping reagent 0.2M H_2SO_4	1 × 25 ml	ready to use; 0.20 mol/l sulphuric acid solution; colourless liquid
Protective film	1	
Plastic dish	2	
Plastic zip-lock bag	1	

The calibrators were calibrated using a WHO 1st IRP 96/668. Exact concentration levels for calibrators and control serum are given on the labels on a lot specific basis.

All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package. Once opened, the components should be used within 2 months. Concentration of preserving agents: <=0.1 %.

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- automatic microplate washer
- microplate reader equipped with 450 nm filter

Safety notes

- as no known test method can offer complete assurance that infections agents are absent, reagents and samples should be handled as if capable of transmitting infectious disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke or apply cosmetics in the laboratory
- do not pipette by mouth
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum as soon as possible to avoid any hemolysis
- do not use sera preserved with sodium azide
- do not use contaminated, hyperlipemic and hyperhemolysed specimens
- the samples with hyperproteinemia and hyperbilirubinemia were not specially tested
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- ★ samples can be stored at 2–8 °C within 72 hours or frozen at -20 °C within 1 month
- no more than one freeze/thaw cycle is allowed

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (20–25 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section "Washing procedure"
- for the description of test procedure with the automated analyzers see section "Automated analyzers"

Washing procedure

Please contact your representative for protocols for recommended washers and procedures.
In general the following protocol is recommended:

- flow-through washing with a volume not less than 300 µl per well is used
- repeat 5 times
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 µl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion **Stability:** 14 days at 18–24 °C or 28 days at 2–8 °C												
Washing solution (concentrated 25-fold), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0
Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0

Test procedure

abia PSA free for the quantitative determination of free prostate specific antigen (fPSA) concentration in human serum

1	Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in plastic zip-lock bag. Do not remove desiccant.
2	Analyse each calibrator, control serum and samples in duplicate. Reserve one or two wells for TMB/substrate solution control (blank). Add 50 µl of calibrators 0 - 5 into appropriate wells. Add 50 µl of control serum into appropriate wells. Add 50 µl of samples to be tested in rest of the wells. The total time should not exceed 10 min.
3	Add 50 µl of sample diluent into each well except blank. Mix the contents of the wells for 30 seconds by careful tapping on the edge of the plate, then cover the plate with protective film.
4	Incubate for 60 minutes at room temperature 20–25 °C.
5	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 300 µl of working washing solution into each well and aspirate. Perform this procedure 5 times. Use double aspiration in the final step where possible.
6	Add 100 µl conjugate into each well except blank. Cover the plate with protective film. ★
7	Incubate for 60 minutes at room temperature 20–25 °C.
8	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 300 µl of working washing solution into each well and aspirate. Perform this procedure 5 times. Use double aspiration in the final step where possible.
9	Add 100 µl of TMB/substrate solution to all the wells. Keep the plates in a dark place for 25 ± 5 minutes at 20–25 °C.
10	Add 150 µl of stopping reagent into each well. Mix gently for 5–10 sec.
11	Read the optical density at 450 nm using a plate reader within 20 minutes after stopping reaction.

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative.
For the instrumentation without established validated protocol follow section "Test procedure" and ensure all requirements described in section "Precautions" are followed.
All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of blank value should be not more than 0.100 at 450 nm.
The absorbance (OD) of calibrator 5 (approx 10 ng/ml) should be greater than 1.300.
Calculated value of control serum should be within established range.

Calculation procedure

1 Calculate the mean optical density of each calibrator duplicate at 450 nm.

2 Calculate the mean optical density of each samples duplicate.

3 Subtract the mean absorbance value of the "blank" from the mean absorbance values of the calibrators, control and serum samples.

4 Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.

5 Read the values of the samples directly off the calibration curve.
If immunoassay software is being used, a 4-parameter curve is recommended.

If a sample reads more than 10 ng/ml then dilute it with calibrator 0. The result obtained should be multiplied by the dilution factor.

Example	OD 1	OD 2	Mean OD - blank (here 0.057)	Value, ng/ml
Calibrator 0	0.054	0.060	0.000	0.00
Calibrator 1	0.244	0.258	0.194	0.50
Calibrator 2	0.413	0.428	0.364	1.00
Calibrator 3	0.755	0.792	0.717	2.00
Calibrator 4	1.652	1.658	1.598	5.00
Calibrator 5	2.653	2.633	2.586	10.00
Sample	0.422	0.426	0.367	1.00

This data is for illustration only and should **not be used** to calculate samples. Each user should obtain his or her own data and standard curve.

★ Performance characteristics

Analytical sensitivity

Limit of detection defined at 0.1 ng/ml.

Specifity	Concentration	Cross reactivty, %
Tumor markers		
Kallikrein-2 (KLK2)	2 000 ng/ml	≤ 0.005
Chorionic gonadotropin (hCG)	93 000 mIU/ml	≤ 0.0018
Alpha-1-Fetoprotein (AFP))	20 000 ng/ml	≤ 0.00115
Carcinoembryonic antigen (CEA)	20 000 ng/ml	≤ 0.00145
Cancer antigen 125 (CA-125)	10 000 U/ml	-
Chemotherapeutic agents		
Doxorubicin-HCl	20 000 ng/ml	≤ 0.002
Methotrexate	100 000 ng/ml	≤ 0.0002
Flutamide	100 000 ng/ml	≤ 0.0002
Cisplatin	60 000 ng/ml	≤ 0.0003
Vincristine	10 000 ng/ml	≤ 0.002

Precision	Mean, ng/ml	SD	CV, %
Intra-assay, max	0.9	0.06	6.6
Inter-assay, max	0.9	0.07	7.8

Accuracy

The assay was compared with an commercially available enzyme immunoassay as a reference test. The total number of specimens was 193. The values ranged from 0.81 to 7.57 ng/ml.

The linear regression curve was calculated: y = 0.93(x) + 0.09 with correlation coefficient 0.95.

Expected fPSA/tPSA ratio

The fPSA/tPSA ratio may be used in differential diagnosis of benign prostate hyperplasia (BPH) and prostate cancer (PCa). The fPSA/tPSA ratio was determined for 81 men with tPSA values between 1.82-36.32 ng/ml. Serum specimens from 59 men with BPH and 22 men with PCa were analysed using the abia PSA free and abia PSA total kit. The choice of a cut off to be used in critical practice depends upon the clinical application, i.e. whether optimized sensitivity or specificity is desired.

fPSA/tPSA ratio	Sensitivity (PCa ≤ cut off)		Specificity (BPH > cut off)	
cut off, %	n = 22	%	n = 59	%
10	9	40.91	57	96.61
15	14	63.64	49	83.10
20	16	72.73	39	66.10

Normal value ranges may vary slightly among different laboratories. It is strongly recommended that each laboratory should establish its own range of expected fPSA/tPSA ratio or takes advantage of the regulations or guidelines of their country, for example the NCCN Clinical Practice Guideline in Oncology "Prostate Cancer Early Detection" Version 1.2023.

Limitations of test

- the assay was validated only for the determination of fPSA in human serum
- only calibrator 0 may be used to dilute any high serum samples. The use of any other reagent may lead to false results
★ – even low total PSA values as well a favourable fPSA/tPSA ratio cannot fully exclude cancer
- the results obtained with this assay should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient
- the assay contains reagents to minimize interference of HAMA and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results
★ – up to 6000 ng/ml fPSA no hook effect was observed in this test
- not intended for newborn screening
★ – test results may be adversely affected by several pre-analytical and clinical factors (e.g. instability of free PSA at 4 °C and room temperature, variable assay characteristics, concominant BPH in large prostates)

★ References

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Key to symbols used

	Manufacturer
IVD	For in vitro diagnostic use
REF	Catalogue number
LOT	Batch code
YYYY-MM-DD	Expiry date
2°C 8°C	Storage temperature limitation
	Do not use if package is damaged
	Do not reuse
Σ n	Sufficient for [n] tests
	Consult Instructions for use
	Caution, consult documents
★	Changes highlighted

Hazard and precautionary statements for certain kit components

Warning

Stopping reagent

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Warning

Conjugate, calibrators 0 - 5, control serum

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.

Attention!
For complete precautionary statements and detailed information see safety data sheets (SDS).

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