abia PSA free

★ REF DK.046.01.6 IVD

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 33kDa serine protease that is predominantly bound to alpha-1-antichymotrypsin (PSA-ACT) and alpha-2-macroglobulin (PSA-AMG) in human serum.

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 utilise the detection of the major PSA-ACT form. However, elevated serum PSA levels have also been attributed to benign prostatic hyperplasia and prostatitis, resulting in a high proportion of false-positive screening results..

One potential solution to this problem is to determine free PSA levels. Preliminary studies have suggested that the percentage of free PSA is lower in patients with prostate cancer than in those with benign prostatic hyperplasia (BPH). Therefore, measuring free serum PSA alongside total PSA can improve the specificity of prostate cancer screening for men with elevated total serum PSA levels, reducing unnecessary prostate biopsies while having minimal effect on cancer detection rates..

According to the NCCN Guidelines, the fPSA/tPSA ratio should be used to detect prostate cancer at an early stage in the following individuals:

- Patients aged 45–75 years who are of average risk, with a total PSA level of >3 ng/ml and/or a very suspicious result from a digital rectal examination.
- Patients aged 40–75 who are at high risk, with a total PSA level of >3 ng/ml and/or an abnormally suspicious result from a digital rectal examination.
- Healthy individuals over 75 years of age with a total PSA level of over 4 ng/ml and/or a highly suspicious result from a digital rectal examination.
- Individuals over 50 years of age who have had a non-suspicious digital rectal examination and whose PSA levels are between 4 ng/ml and 10 ng/ml.

Testing laboratories are strongly recommended to take advantage of the regulations or guidelines of their country. The fPSA/tPSA ratio should be used as an additional marker to aid physicians, but should never be used in isolation for decision-making purposes (see also 'Limitations of the test')..

Principle of the test

abia PSA free is a two-step immunoassay, based on the “sandwich” method principle.

The assay system uses high-affinity, specific monoclonal antibodies (enzyme-conjugated and immobilised) that target a distinct antigenic determinant on the intact fPSA molecule.

The test sample reacts with both antibodies simultaneously, resulting in the fPSA molecules being "sandwiched" between the solid phase and the enzyme-linked antibodies.

The unbound components are the removed by washing. After the addition of a solution containing TMB and hydrogen peroxide, the wells containing the bound conjugate develop a blue colour, which turns yellow once the reaction is 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 International Standard, NIBSC 17/102. The exact concentration levels for the calibrators and control serum are given on the labels on a lot-specific basis.

All components are stable until the kit's eypiry date when stored at 2–8 °C in a tightly sealed package. The expiry date is indicated on the package.
Once opened, the components should be used within two 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

- Since no known test method can provide complete assurance that infectious agents are absent, reagents and specimens should be handled as if they were capable of transmitting infectious disease; any equipment that comes into direct contact with specimens and reagents should be considered as contaminated.
- Do not eat, drink, smoke or apply cosmetics in the laboratory.
- Do not pipette by mouth.
- Avoid contact of reagents and specimens with the skin and mucous membranes; wear lab coats and disposable gloves when handling; wash hands thoroughly after use.
- Avoid spilling samples or solutions that contain them. Wipe up any spills immediately and de-contaminate any affected surfaces.
- All materials that have come into contact with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with local 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 lids 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 samples according to current practice.
- Use serum for testing; performance of the test has not been evaluated with other biological fluids.
- Separate clots or red cells from serum as soon as possible to avoid haemolysis.
- Do not use sera preserved with sodium azide.
- Do not use contaminated, hyperlipaemic and hyperhaemolysed samples
- Specimens with hyperproteinemia and hyperbilirubinemia have not been specifically tested.
- Samples with observable particulate matter should be clarified by centrifugation prior to testing.
- Suspended fibrin particles or aggregates may cause reactive results.
- Do not heat the samples.
- Specimens may be stored at 2–8 °C for 72 hours or frozen at -20 °C for 1 month.
- No more than one freeze-thaw cycle is allowed.

Procedural notes

- Allow reagents to stabilise at room temperature (20 - 25 °C) for at least 30 minutes before use.
- Check the appearance of the reagents.
- Loss of vacuum in the coated plate bag will not affect the performance of the test.
- Check pipettes and other equipment for accuracy and correct operation.
- The washing procedure is a critical step; see the "Washing procedure" section for detailed washer settings.
- For a description of the test procedure with the automated analysers see the "Automated analysers" section

Washing procedure

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

- Use flow-through washing with a volume of not less than 300 µl per well.
- Repeat 5 times.
- Do not allow the wells to dry out during the assay procedure.
- Ensure that no liquid is left in the well (use double aspiration in the last step if possible)
- Avoid tapping out the plate.
- A residual volume of less than 10 µl is not critical for subsequent steps in the assay procedure.
- If a microplate washer is used, clean the wash head frequently to avoid 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 is a test for the quantitative determination of the concentration of free prostate-specific antigen (fPSA) in human serum.

1	Unpack the required number of coated strips. Return the unused strips to the pouch and reseal the foil-lined package in plastic zip-lock bag. Do not remove the 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 to the appropriate wells. Add 50 µl of Control serum to the appropriate wells. Add 50 µl of the samples to be tested to the remaining wells. The total time should not exceed 10 minutes.
3	Add 50 µl of sample diluent to each well except the blank. Mix the contents of the wells by careful tapping the edge of the plate for 30 seconds, 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 avoid spillage. Aspirate the contents of all wells into a biohazardous waste container (containing disinfectant). Add at least 300 µl of Working Washing solution into each well and aspirate. Repeat this procedure 5 times. If possible, use double aspiration in the last step.
6	Add 100 µl conjugate to each well except the 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 avoid spillage. Aspirate the contents of all wells into a biohazardous waste container (containing disinfectant). Add at least 300 µl of Working Washing solution to each well and aspirate. Repeat this procedure 5 times. If possible, use double aspiration in the last step.
9	Add 100 µl of TMB/substrate solution to all the wells. Allow the plates to stand for 25 ± 5 minutes at 20–25 °C in the dark.
10	Add 150 µl of Stopping reagent to each well. Mix gently for 5–10 seconds.
11	Read the optical density at 450 nm using a plate reader within 20 minutes after stopping the reaction.

Automated analysers

Validated protocols for automated analysers can be obtained from your representative.

For instruments without an established validated protocol follow the "Test procedure" section and ensure all requirements described in the "Precautions" section are followed.

All protocols for automated analysers must be fully validated before use.

Calculation and interpretation of the results

Assay validation

The results of an assay are valid if the following criteria are met for the controls:

The absorbance (OD) of the blank should be ≤ 0.100 at 450 nm.
The absorbance (OD) of Calibrator 5 (approx. 45 ng/ml) should be ≥ 1.300.
The calculated value for the control serum should fall within the specified range.

Calculation procedure

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

2 Calculate the average optical density of each sample duplicate.

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

4 Plot 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 sample values directly from the calibration curve.
If immunoassay software is being used, a 4-parameter curve is recommended.

If a sample reads more than 10 ng/ml, dilute it with Calibrator 0 and then multiply the result 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

These data are for illustrative purposes only and should not be used for sample calculations. Each user should generate their own data and standard curve.

Performance characteristics

Analytical sensitivity

The limit of detection is defined as 0.1 ng/ml.

Specificity	Concentration	Cross reactivty, %
Tumour markers		
Kallikrein-2 (KLK2)	2 000 ng/ml	≤ 0.00500
Chorionic gonadotropin (hCG)	93 000 mIU/ml	≤ 0.00180
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	≤ 0.0020
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	≤ 0.0020

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 a commercially available enzyme immunoassay, which was used as the reference test. A total of 193 specimens were analysed. The values ranged from 0.81 to 7.57 ng/ml.

A linear regression curve was calculated, with the equation y = 0.93(x) + 0.09 and a correlation coefficient of 0.95.

Expected fPSA/tPSA ratio

The free-to-total prostate-specific antigen (fPSA/tPSA) ratio can be used to differentiate between benign prostatic hyperplasia (BPH) and prostate cancer (PCa). The ratio was determined for 81 men with tPSA values ranging from 1.82 to 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 kits. The choice of cut-off point for critical practice depends on the desired clinical outcome, i.e. whether sensitivity or specificity is prioritised.

fPSA/tPSA ratio cut off, %	Sensitivity (PCa ≤ cut off) n = 22	%	Specificity (BPH > cut off) 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 between different laboratories. It is strongly recommended that each laboratory establishes its own expected fPSA/tPSA ratio range, or takes advantage of the relevant country-specific regulations or guidelines, such as the NCCN Clinical Practice Guideline in Oncology 'Prostate Cancer Early Detection' Version 1.2023.

Limitations of the test

- The assay has only been validated for determining fPSA in human serum.
- Only Calibrator 0 can be used to dilute highly concentrated serum samples. Using any other reagent may lead to false results.
- Even low total PSA values and a favourable fPSA/tPSA ratio cannot rule out cancer.
- Results obtained with this assay should never be used as the sole basis for clinical diagnosis. Any laboratory result is only part of a patient's overall clinical picture.
- The assay contains reagents to minimise interference from human anti-mouse antibodies (HAMA) and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results.
- No hook effect was observed at fPSA concentrations of up to 6,000 ng/ml.
- This test is not intended for newborn screening.
- Test results may be adversely affected by several pre-analytical and clinical factors, such as the instability of free PSA at 4 °C and room temperature, variable assay characteristics and concomitant benign prostatic hyperplasia (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
	Do not use if package is damaged
	Do not reuse
Σ n	Sufficient for [n] tests
	Consult Instructions for use
★	Changes highlighted
★ YYYY-MM-DD	Date of manufacturing
YYYY-MM-DD	Expiry date
2°C 8°C	Storage temperature limitation

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 + P338 + P351	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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