abia PSA total

★ REF DK.011.01.6 IVD

Note: Changes highlighted ★

abia

Intended use

abia PSA total is an enzyme immunoassay for the quantitative determination of total prostate specific antigen (tPSA) concentration in human serum or plasma.

For professional use only.

Clinical value

Human prostate specific antigen (PSA) is a serine protease and a single chain glycoprotein with a molecular weight of approximately 33 kDa, containing 7% carbohydrate by weight.

PSA is immunologically specific to prostatic tissue. It is present in normal, benign hyperplastic and malignant prostate tissue, as well as in metastatic prostate carcinoma, prostate fluid and seminal plasma. PSA is not present in any other normal tissue obtained from men, nor is it produced by cancers of the breast, lung, colon, rectum, stomach, pancreas or thyroid. Furthermore, PSA is functionally and immunologically distinct from prostatic acid phosphatase (PAP). Elevated serum PSA concentrations have been reported in patients with prostate cancer, benign prostatic hypertrophy or inflammatory conditions affecting adjacent genitourinary tissues. However, it is not present in men with non-prostatic carcinoma, apparently healthy women, or women with cancer. Reports have suggested that serum PSA is one of the most useful tumour markers in oncology. It may serve as an accurate marker for assessing the response to treatment in patients with prostate cancer.

Therefore, measuring serum PSA concentrations can be an important way of monitoring patients with prostate cancer and determining the effectiveness of surgery or other therapies, both potential and actual.

Principle of the test

abia PSA total is a one-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 tPSA molecule.

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

The unbound components are then 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 tPSA 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-PSA Ab
Conjugate	1 × 12 ml	ready to use; HRP-labelled monoclonal anti-PSA; transparent or slightly opalescent pink liquid
Calibrator 0	1 × 2.0 ml	protein based buffer not containing tPSA; pale yellow liquid
Calibrator 1	1 × 0.75 ml	protein based buffer containing tPSA in concentration approx. 1.50 ng/ml; pale yellow liquid
Calibrator 2	1 × 0.75 ml	protein based buffer containing tPSA in concentration approx. 3.75 ng/ml; pale yellow liquid
Calibrator 3	1 × 0.75 ml	protein based buffer containing tPSA in concentration approx. 7.50 ng/ml; pale yellow liquid
Calibrator 4	1 × 0.75 ml	protein based buffer containing tPSA in concentration approx. 15.00 ng/ml; pale yellow liquid
Calibrator 5	1 × 0.75 ml	protein based buffer containing tPSA in concentration approx. 45.00 ng/ml; pale yellow liquid
Control serum	1 × 0.75 ml	protein based control containing tPSA; pale yellow liquid
Washing solution (concentrated 25-fold)	1 × 50 ml	phosphate saline buffer; colourless or pale yellow liquid
TMB/substrate solution	1 × 14 ml	ready to use; citric acid buffer containing TMB and H_2O_2; colourless to pale blue 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 17/100. 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 expiry 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

- The human material used in the preparation of the calibrators and control serum has been tested and found to be negative for hepatitis B surface antigen (HBsAg), hepatitis C virus antibodies, and human immunodeficiency virus antibodies (HIV-1 and HIV-2).
- 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 decontaminate 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 the 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 heparin/EDTA plasma or serum for testing; performance of the test has not been evaluated with other biological fluids.
- Separate clots or red cells from serum or plasma 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 hyperproteinaemia and hyperbilirubinaemia 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 48 hours or frozen at -20 °C.
- 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 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	6	9	12	15	18	21	24	27	30	33	40
Purified water, ml	72	144	216	288	360	432	504	576	648	720	792	960

Test procedure

abia PSA total is used for the quantitative determination of total prostate-specific antigen (tPSA) concentration in human serum or plasma.

1 Unpack the required number of coated strips. Return the unused strips to the pouch and reseal the foil-lined package in the plastic zip-lock bag. Do not remove the desiccant.

2 Analyse each calibrator, control serum and sample in duplicate. Reserve one or two wells for the TMB/substrate solution control (blank).
Add 25 µl of Calibrators 0 - 5 to the appropriate wells.
Add 25 µl of Control serum to the appropriate wells.
Add 25 µl of the samples to be tested to the remaining wells.
The total time should not exceed 10 minutes.

3 Add 100 µl conjugate to each well except the blank.
Mix the contents of the wells by carefully tapping the edge of the plate for 30 seconds, then cover the plate with protective film.

4 Incubate for 75 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 of TMB/substrate solution to all the wells. Allow the plates to stand for 25 ± 5 minutes at 20–25 °C in the dark.

7 Add 150 µl of Stopping reagent into each well. Gently mix gently for 5–10 seconds.

8 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 that all requirements described in the "Precautions" section are met.

All automated analyser protocols 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 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 greater than 45 ng/ml, dilute with Calibrator 0 and multiply the result by the dilution factor.

Example	OD 1	OD 2	Mean OD - blank (here 0.046)	Value, ng/ml
Calibrator 0	0.043	0.049	0.000	0.00
Calibrator 1	0.181	0.181	0.135	1.50
Calibrator 2	0.345	0.358	0.306	3.75
Calibrator 3	0.656	0.662	0.613	7.50
Calibrator 4	1.226	1.234	1.184	15.00
Calibrator 5	2.594	2.624	2.563	45.00
Sample	0.363	0.389	0.330	4.00

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 limits of detection (LoD) and quantification (LoQ) were determined in accordance with the CLSI EP-17A2 protocol.

The limit of detection is defined as 0.282 ng/ml. The limit of quantification is defined as 0.39 ng/ml.

Specificity	Concentration	Cross reactivity, %
Kallikrein-2 (KLK2)	2 000 ng/ml	0.0280
Chorionic gonadotropin (hCG)	20 µg/ml	0.0040
Carcinoembryonic antigen (CEA)	20 µg/ml	0.0066
Alpha-1-Fetoprotein (AFP)	20 µg/ml	0.0032

Precision	Mean, ng/ml	SD	CV, %
Intra-assay, sample 1	4.20	0.25	6.00
Intra-assay, sample 2	13.10	0.60	4.60
Intra-assay, sample 3	22.30	1.01	4.50
Inter-assay, sample 1	4.20	0.31	7.50
Inter-assay, sample 2	13.10	0.90	6.90
Inter-assay, sample 3	22.30	1.62	7.20

Linearity

Thirteen serum samples, ranging from 1.13 to 39.31 ng/ml, were diluted with Calibrator 0 and tested in triplicate. The measured concentrations were within ±10% of the expected concentrations.

Accuracy

The assay was compared with a commercially available enzyme immunoassay, which was used as the reference test. A total of 234 specimens were analysed. The values ranged from 0.28 to 37.07 ng/ml. The least squares regression equation and the correlation coefficient were computed for abia PSA total in comparison with the reference method.

The least squares regression analysis yielded the equation y = 1.10x + 0.09, with a correlation coefficient of 0.97.

Expected normal values	Range, ng/ml	
‹ 40 years	0.01	1.51
40 - 49 years	0.00	2.27
50 - 59 years	0.02	2.89
› 60 years	0.29	4.96

It is known that normal tPSA levels increase with age. Age-specific reference ranges are used to diagnose prostate cancer.

Normal value ranges may vary slightly between different laboratories. Therefore, it is strongly recommended that each laboratory determines its own expected normal value range.

Limitations of the test

- Only Calibrator 0 should be used to dilute highly concentrated serum samples. Using any other reagent may lead to inaccurate results.
- 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 tPSA concentrations of up to 3,000 ng/ml.
- Not intended for newborn screening.

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 + P 352	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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