

INTENDED USE

The D.D. total PSA ELISA is used for the quantitative determination of total prostate specific antigen (t-PSA) in human serum or plasma samples. The determination of t-PSA levels is used to estimate the risk of prostate carcinoma in men in conjunction with digital rectal examination (DRE) or to monitor the effectiveness of prostate carcinoma treatment in patients.

INTRODUCTION

Prostate cancer is the most frequent type of cancer found in man and is the second cause of death due to cancer in males. Until recently, digital rectal examination (DRE) was frequently used as only diagnostic modality for the detection of early stages of prostate cancer. In the recent years the determination of serum PSA levels has become the most accepted method to improve the diagnostic specificity of DRE. Although PSA is a tissue specific protein and is not solely tumor specific, it has become the most important marker for prostate carcinoma, showing a better specificity than other biochemical markers used in this context (PAP, total alkaline phosphatase, carcinoembryonic antigen, etc.)

In 1979, Wang et al isolated a specific antigen for normal prostate tissue and called this protein PSA. As demonstrated by immunohistological studies, PSA is localized in the cytoplasm of prostate acinar cells, ductal epithelium and in the secretion on the ductal lumina, present in normal, benign hyperplastic and malignant prostate tissues as well metastatic prostate cancer and in seminal plasma. If the structural integrity of the prostate is disturbed and/or the gland size is increased, the amount of PSA in the blood plasma may become elevated. An elevation of PSA levels to values higher than 3-4 ng/ml has been reported for patients with either benign prostatic hypertrophy (BPH) or prostate carcinoma. At this threshold follow-up examinations that allow to differentiate between these two conditions are recommended.

The determination of PSA serum levels is not only important for the screening of patients for prostate cancer, but also for monitoring patients that have been treated for this disease. Here regular PSA measurements are an important tool to examine the potential and actual effectiveness of surgery or other therapies. An increase of PSA in patients after radical prostatectomy or radiotherapy may allow an earlier discovery of residual or recurrent carcinoma.

PRINCIPLE OF THE ASSAY

This assay is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with an antibody, directed towards an epitope of an antigen molecule (PSA). An aliquot of patient serum is incubated in the coated well with enzyme conjugated second antibody (E-Ab), directed towards a different region of the antigen molecule. After incubation the unbound E-Ab is washed off. The amount of bound E-Ab is proportional to the concentration of antigen in the sample. After adding the substrate solution, the intensity of colour developed is proportional to the antigen concentration in the sample. The measured ODs of the standards are used to construct a calibration curve against which the unknown samples are calculated.

MATERIALS PROVIDED WITH THE KIT

Each kit contains reagents sufficient for 96 determinations.

A) Microtiterplate:

12 modules with 8 wells each= 96 determinations

B) 5 PSA-Standards:

Ready-to-use reagents (0.50 ml) at the following concentrations:

1) 25ng PSA/mL; 2) 12.5ng PSA/mL; 3) 6.25ng PSA/mL;
4) 3.1ng PSA/mL; 5) 1.56ng PSA/mL

Preservative: Thimerosal 0.02% Kathon 0.1%.

The standards are calibrated against WHO 96/670.

C) Zero Standard/ Sample Diluent:

Ready-to-use reagent (10ml)

D) Control:

Ready-to-use reagent (0.50 ml); for concentration see label of kit

Preservative: Thimerosal 0.02% Kathon 0.1%.

E) PSA Conjugate:

Ready to use conjugate (12 ml)

F) TMB – Substrate:

Ready-to-use reagent (12ml)

Contains TMB (tetramethylbenzidine) and H₂O₂

G) Stop Solution:

Ready-to-use reagent (12ml)

Contains sulphuric acid

MATERIAL REQUIRED BUT NOT PROVIDED

- Precision micropipettes (volume: 25µl and 100µl) with disposable tips
- Distilled water
- ELISA photometer with 450nm- and 630nm-filters
- Timer with 60 min. range or higher
- Microplate washer (optional)
- Vortex or similar mixing tools
- Container for the proper handling of waste and samples after use

STORAGE AND STABILITY

- Store the kit and components at +2 to+ 8°C
- Bring to room temperature (18-25°C) at least 30 minutes before use. After use put back into the refrigerator. Avoid long time storage at room temperature.
- Do not use the kit or components after the expiry date. For expiry date of the original packed kit see kit label.
- Close the bottles immediately after use.
- Store the plate incl. desiccant in the provided zip-lock pouch. Modules that are not used should always be stored under this condition.
- Ensure that kit components do not freeze.

PRECAUTIONS

- ELISA kits are only for in vitro diagnostic use by professionals.
- Serum and plasma samples should be treated as potentially infectious materials. Wear gloves and proper laboratory attire when handling sample materials. Do not eat, drink or smoke in areas where specimen or kit reagents are handled. Do not pipette with the mouth. In case of skin contact, wash with a germicidal soap and copious amounts of water. Seek medical advice if indicated.
- The PSA standards and controls are of human origin. They have been tested and confirmed negative for HIV, HBsAg and HCV. However, all standards should be treated as potential biohazards.
- Due to the potentially infectious character of samples and kit components all materials that have come in contact with these materials should be sterilized and disposed of according to local regulations. This also includes the liquid waste.
- The assay reagents contain preservatives, TMB, H₂O₂ or sulphuric acid and may be harmful if ingested. A direct skin or mucosa contact should be avoided. In case of skin contact, wash thoroughly with water and seek medical attention if required.
- The stop solution contains H₂SO₄. Since the H₂SO₄ used to terminate the color reaction is corrosive, the instrumentation employed to dispense it should be thoroughly cleaned after use.
- Do not interchange reagents from different LOT# or different suppliers.
- Avoid reagent or sample carry-over by using fresh tips for solutions and samples.
- Do not use test kit if zip lock pouch or bottles have been damaged.

GUIDELINE FOR SAMPLE COLLECTION; PREPARATION AND STORAGE

Sample collection

Blood samples are collected by veinpuncture. As different factors could influence the PSA level in blood, doctors should ensure that the patient has avoided the following conditions before taking the blood sample.

The following conditions may lead to an increase of PSA levels

- biking
- sexual intercourse (ejaculation)
- Manipulation of the prostate during medical examinations like DRE, transrectal prostatic ultrasound etc.
- Prostatitis
- Liver dysfunction

The following conditions may lead to a decrease of PSA levels

- Intake of 5-alpha-reductaseinhibitors, antiandrogens, or GnRH analoga

Sample preparation

The preparation of serum or plasma samples is performed according to standard techniques. Serum or plasma should be prepared as soon as possible to avoid hemolysis and to improve the stability of PSA.

Storage of samples

For the assay either fresh serum or plasma samples can be used. If not used immediately they can be stored at 2-8°C for 1 week. In case of longer storage, freeze at -20°C. A repeated freezing and thawing of samples should be avoided.

Note

- Highly lipemic or hemolytic samples can give incorrect analytical results.
- Samples must be free of microbial contaminations.
- Samples containing high titers of rheumatoid factor and human anti-mouse antibodies (HAMA) could give erroneous results.

ASSAY PROCEDURE

Note: It is highly recommended to perform all measurements as duplicates. An independent standard curve should be made for each series of measurements. For best results it is important that the solutions are always added to the wells in the same order to minimize variations in incubation times.

- 1) Prior to use bring all reagents, standards, controls, and samples to room temperature (18-25°C).
- 2) Check that all components are not expired and take care that bottles and plate (inclusive pouch) are not damaged.
- 3) Format the required microplate wells. Keep in mind that all measurements should be performed as duplicate. Document position of wells and respective samples, standards and controls to ensure later identification. Put any unused microwell modules back into the zip lock bag with the desiccant, seal bag and store at 2-8 °C.
- 4) Pipette 25 µl of standards, controls or samples into each well. Samples with an expected PSA value higher than 25 ng/ml should be diluted with the sample diluent.
- 5) Incubate 5 min at room temperature (18-25°C)
- 6) Add 100 µl of PSA conjugate into each well
- 7) Mix by moving plate on the table (10sec)
- 8) Incubate 1h at room temperature (18-25°C)
- 9) Remove solution from the wells by aspirating the liquid or by decanting it. If decanting, tap plate on adsorbent paper to remove residual liquid.
- 10) For washing fill plate with distilled water and wait 15 sec before removing the distilled water; wash 5x to 6x.

We recommend the following procedure: wash wells 6-times with 250µL / well distilled water. Preferably use an automated washing procedure. If washing manually take care that the washing solution remains in each well for the same time. This is necessary to receive lowest possible CV-values.

- 11) Pipette 100µl TMB-substrate solution into each well
- 12) Incubate 20min at room temperature (18-25°C)
- 13) Add 100µl/well stop solution (same order as substrate solution)
- 14) Read absorbencies (OD) at 450 nm (blanking 630nm)

Results:

- 1) Calculate the mean absorbance for each duplicate.
- 2) Subtract the absorbance value of the zero standard from the mean absorbance values of standards, control and samples.
- 3) Draw the standard curve on lin-lin or log-log graph paper by plotting absorbance values of standards against appropriate PSA concentrations or use a proper software of the ELISA reader used.
- 4) Read off or calculate the PSA concentrations for the control and the samples.

VALIDITY OF THE ASSAY

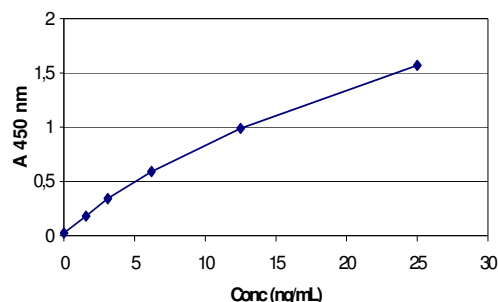
- 1) The OD 450 nm of the blanking well is lower than 0.150. Higher values indicate a chromogen/substrate contamination. In such a case, repeat the assay carefully checking the reagent.
- 2) The OD 450 nm of the highest standard (25 ng/mL) must be higher than 0.700. Lower values indicate kit or control decay. In such a case, check the expiry date of the kit before repeating the assay.
- 3) The control provided should not differ by more than 15% from the concentration stated on the label of the vial if run at least in duplicate.
- 4) Worksheet and standard curve of typical assay: Not to be used for calculation of actual test results.

Example:

Wells	Identity	A 450 nm		Conc. ng/mL
1-2	St 0ng/mL	0.022	0.023	
3-4	St 1,56ng/mL	0.178	0.180	
5-6	St 3,10ng/mL	0.337	0.342	
7-8	St 6,20ng/mL	0.611	0.568	
9-10	St 12,50ng/mL	0.990	0.984	
11-12	St 25,00ng/mL	1.574	1.562	
13-14	control	0.421	0.400	3.98

Lot 30902.2

PSA



Note that the absolute OD values for the standards might vary due to temperature influences or age of the conjugate. As long as the OD values form a standard curve and remain within the specifications and the control shows the expected value, results for unknown PSA samples are valid.

QUALITY CONTROL

- It is recommended that internal controls are used in every assay in duplicate. Control results should be within established ranges and should preferably represent low, medium, and high concentrations.
- The risk for the patient is mainly due to false negative results around the reshold (cut-off) value of PSA <4.0 ng/mL. It is therefore highly recommended to validate the kit and laboratory via external trials (e.g. DGKC).

EXPECTED VALUES LIMITATIONS OF THE PROCEDURE

The generally recommended threshold for follow-up examinations is:

Cut-off value: 3.0-4.0ng PSA /mL

Healthy men generally have a PSA concentration lower than 4.0 ng/mL. If the PSA concentration is equal or higher than 4.0 ng/ml follow-up examinations are highly recommended. This PSA concentration indicates an elevated risk for prostate cancer but might also be caused by BPH. Please note that that the 4 ng/ml threshold is only a guideline value. In the literature it is discussed that modifications according to age and ethnological background might be useful e.g. that for younger men the threshold should be lower than for older men. If possible, it is recommended for each laboratory to establish its own specific values that take into consideration a population indigenous to the area where the laboratory is located.

It is important to keep in mind that some prostate tumors do not cause elevated PSA levels so that PSA measurements should never replace DRE but should only be used in conjunction with DRE.

As elevated PSA levels might also be caused by non-cancerous conditions follow examinations might try to increase the diagnostic specificity of t-PSA values. In the literature PSA density, PSA velocity and the ratio of f-PSA to t-PSA are discussed to improve discrimination between cancerous and non-cancerous conditions and might be used to reduce unnecessary prostate biopsies. But only a prostate biopsy can finally show if a prostate carcinoma is present or not.

Note: PSA values can only be used to estimate the cancer risk. They should always be interpreted in conjunction with other clinical findings and should not be used as a sole basis for prostate cancer diagnosis.

PERFORMANCE CHARACTERISTICS

1) Detection limit

The limit of detection for this kit is 0.2 ng/mL

2) Precision

Intra- and inter-assay precisions were established by analysing three patient sera of different PSA concentrations. The results are shown in Tables 1 and 2.

Table 1. Intra-assay precision

Patients	Number of replicates	Mean ng/mL	SD ng/mL	CV %
1	24	12.52	0.65	6.0
2	24	3.44	0.13	3.9
3	32	0.83	0.07	8.8

Table 2. Inter-assay precision

Patients	Number of replicates	Mean ng/mL	SD ng/mL	CV %
1	4	12.28	0.82	6.7
2	4	3.33	0.266	7.98

3) Recovery

A known amount of PSA was added to three patient sera and the quantities recovered were measured. The results are shown in Table 3.

Table 3. Recovery

sample	Expected value (ng/mL)	Observed value (ng/mL)	Recovery %
1	6.30	6.40	102
2	4.67	4.56	98
3	10.10	10.91	108

4) Specificity

The antibodies used in this kit are highly specific for total PSA (free PSA & PSA-ACT-complex), with a relatively low cross-reactivity to other proteins and polypeptides, lipids or chemotherapeutic agents that might be present in patient samples.

Table 4. Specificity

Antigens	Amount added	Cross reaction
Proteins		
AFP	10 µg/mL	No
CEA	10 µg/mL	No
HCG	10 µg/mL	No
Lactalbumin	10 µg/mL	No
PAP	1 µg/mL	No
Interfering substances		
Bilirubin	0.2 mg/mL	No
Hemoglobin*	0.1 mg/ml	No
Triglyceride	15 mg/mL	No
Chemotherapeutic Agents		
Cyclophosphamid	800 µg/mL	No
Doxorubicin * HCl	20 µg/mL	No
Diethylstilbestrol	2 µg/mL	No
Flutamide	10 µg/mL	No
Methotrexate	50 µg/mL	No

* at higher concentration hemoglobin results in too high OD values, hemolytic samples should thus be avoided.

5) High dose hook effect

The assay was tested for a high dose hook effect. Up to a PSA concentration of 2000 ng/mL no hook effect was observed. Please note that if the OD is out of the standard range for highly concentrated samples, the sample must be diluted before the next measurement to obtain correct results.

6) Correlation

The D.D. total PSA ELISA was compared with the Roche ElecSys total PSA:

$$Y = 0.9644 x + 0.0741$$

In a second study the D.D. t-PSA ELISA was compared to another CE registered PSA ELISA:

$$Y = 1.001x, R^2 = 0.9704$$

7) Calibration

The D.D. total PSA ELISA is calibrated against WHO Standard 96/670.

SUGGESTED READING

- Fritsche HA und RJ. Babalan Clin Chem (1993) Vol: 39: 1529-1529 Analytical performance goals for measuring prostate-specific antigen:
- Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaft (AWMF): Leitlinien der Deutschen Urologen: PSA-Bestimmung in der Prostatakarzinomdiagnostik (2003) <http://www.uni-duesseldorf.de/WWW/AWMF/II/uro-36v.htm> (Stand Juli 2003)
- Hammerer P. and Huland H., Der Onkologe (1996), Vol 2: 218-223 Früherkennung des Prostatakarzinoms. Onkologe
- Milford Ward A. et al., Ann Clin Biochem (2001), Vol 38: 633-651 Prostate specific antigen: biology, biochemistry and available commercial assays.
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- Lange P et al., J Urol (1989) Vol 141:873 The value of serum prostate-specific antigen determinations before and after radical prostatectomy,
- Akdas et al. British J Uro (1997) Vol 79: 920-923 The role of free prostate specific antigen in the diagnosis of prostate cancer
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Symbols used



In-Vitro Diagnostic Kit



Content



Lot number



Do not expose to sunlight



Expiry date



Storage temperature



Read user instructions carefully

Notified Body:

MDC, Medical Device Certification GmbH, Stuttgart,
Identification number: 0483

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