

REVISED 13 SEPT. 2011 RM (VERS. 8.1)

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

The free PSA ELISA is for determination of free prostate specific antigen (t-PSA) in human serum or plasma samples. The determination of f-PSA levels is generally used in conjunction with a total PSA (t-PSA) measurement to determine the ratio between f-PSA and t-PSA. This ratio helps to estimate the risk for prostate cancer and to discriminate between elevated t-PSA levels caused by cancerous or non-cancerous conditions.

2 PRINCIPLE OF THE TEST

This f-PSA ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA). The microtiter wells are coated with an anti-f-PSA monoclonal antibody, directed towards an epitope of an antigen molecule. An aliquot of sample 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.

3 MATERIALS PROVIDED WITH THE KIT

Each kit contains reagents sufficient for 96 determinations.

1. **Microtiterwells**, 12 x 8 (break apart) strips, 96 wells.
Wells coated with anti-free PSA antibody (monoclonal).
2. **Zero Standard/Sample Diluent**, 1 vial, 10.0 mL, ready to use.
Contains non-mercury preservative.
3. **Standard (Standard 1-5)**, 5 vials, 0.5 mL, ready to use;
Concentrations: 0.75 – 1.5 – 3.0 – 6.0 – 12.0 ng/mL
The standards are calibrated against NIBSC (WHO) Standard 96/668
Contain non-mercury preservative.
4. **Control**, 1 vial, 0,5 mL each, ready to use;
For control values and ranges please refer to vial label or QC-Datasheet.
Contain non-mercury preservative.
5. **Assay Reagent**, 1 vial, 6 mL, ready to use;
Contains non-mercury preservative.

REVISED 13 SEPT. 2011 RM (VERS. 8.1)

6. **Enzyme Conjugate**, 1 vial, 6 mL, ready to use,
Anti-PSA antibody conjugated to horseradish peroxidase;
Contains non-mercury preservative.
7. **Substrate Solution**, 1 vial, 12 mL, ready to use,
Tetramethylbenzidine (TMB).
8. **Stop Solution**, 1 vial, 14 mL, ready to use,
contains 0.5 M H₂SO₄.
Avoid contact with the stop solution. It may cause skin irritations and burns.
9. **Wash Solution**, 1 vial, 25 mL (40X concentrated),
Dilute with distilled water before use.
see „Reagent Preparation“.

4 MATERIAL REQUIRED BUT NOT PROVIDED

- Precision micropipettes (volume: 25 µL and 100 µL) with disposable tips
- Distilled water
- ELISA photometer with 450 nm- and 630 nm-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

5 STORAGE AND STABILITY

- Store the kit and components at 2 °C to 8 °C
- Bring to room temperature (18 °C - 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.

REVISED 13 SEPT. 2011 RM (VERS. 8.1)

6 PRECAUTIONS

- ELISA kits are only for research use only 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.

7 GUIDELINE FOR SAMPLE COLLECTION; PREPARATION AND STORAGE

7.1 Sample collection

Blood samples are collected by venipuncture. As different factors could influence the PSA level in blood, researchers should be aware that the following conditions influence results 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. Please note that especially DRE might lead to an increase of f-PSA only, so that PSA carcinomas might be overlooked
- Prostatitis
- Liver dysfunction

The following conditions may lead to a decrease of PSA levels

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

REVISED 13 SEPT. 2011 RM (VERS. 8.1)

7.2 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.

7.3 Storage of samples

For the assay either fresh serum or plasma samples can be used. F-PSA is not as stable as t-PSA.

Literature (Thomas, 2008) recommends to measure samples within 24 hours. 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.

8 ASSAY PROCEDURE

8.1 Reagent preparation

Before starting the assay the wash buffer must be diluted to the right concentration.

Per well approximately 2 mL of diluted buffer are needed. Calculate the volume of buffer required for the testing.

Take 1/40 of the volume wash buffer concentrate and dilute with 39/40 of the volume distilled water.

8.2 Test 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 f-PSA value higher than 12 ng/ml should be diluted with the sample diluent.
5. Add 50 µL of Assay Reagent into each well and mix by moving plate on the table (10 sec.)
6. Incubate 1 h at room temperature (18-25°C)
7. Add 50 µL of conjugate into each well and mix by moving plate on the table (10 sec.)
8. Incubate 1 h 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.

REVISED 13 SEPT. 2011 RM (VERS. 8.1)

10. For washing fill plate with diluted wash buffer and wait 10sec before removing the buffer; repeat wash 5 times (for a total of 6 times)
We recommend the following procedure: wash wells 6-times with 250-300 μ L/well diluted wash buffer. Preferably use an automated washing procedure. If washing manually, take care that the wash buffer remains in each well for the same time. This is necessary to receive lowest possible CV-values.
11. Pipette 100 μ L TMB-substrate solution into each well
12. Incubate 15 min at room temperature (18-25°C)
13. Add 100 μ L/well stopping solution (same order as substrate solution)
14. Read absorbencies (OD) at 450 nm (blanking 630nm)

8.3 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.
4. Read off the f-PSA concentrations for the control and the samples.

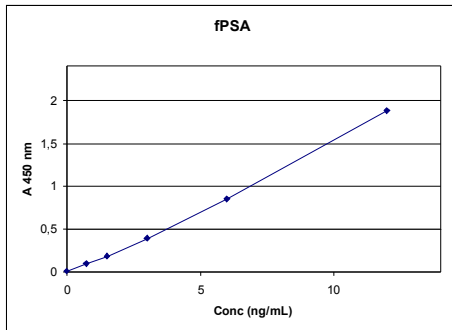
9 VALIDITY OF THE ASSAY

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 (12 ng/mL) must be higher than 0.9. 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

REVISED 13 SEPT. 2011 RM (VERS. 8.1)

Wells	Identity	A 450 nm		Conc. ng/mL
1-2	St 0.00ng/mL	0.011	0,009	
3-4	St 0.75ng/mL	0.109	0.096	
5-6	St 1.5ng/mL	0.185	0.179	
7-8	St 3.0ng/mL	0.393	0.392	
9-10	St 6.0ng/mL	0.871	0.837	
11-12	St 12.0ng/mL	1.901	1.844	
13-14	control	0.218	0.231	2.05



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.

10 QUALITY CONTROL

- It is recommended that internal controls are used in every assay.
- Control results should be within established ranges and should represent preferably low, medium and high concentrations.

10.1 Calibration

The free PSA ELISA (EIA-4189) is calibrated against WHO Standard 96/668.

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10.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point 14.2. are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

11 SUGGESTED READING

1. Fritsche HA und RJ. Babalan Clin Chem (1993) Vol: 39: 1529-1529 Analytical performance goals for measuring prostate-specific antigen:
2. Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaft (AWMF): Leitlinien der Deutschen Urologen: PSA-Bestimmung in der Prostatakarzinomdiagnostik (2003) <http://www.uni-duesseldorf.de/WWW/AWMF/II/uro1-36v.htm> (Stand Juli 2003)
3. Hammerer P. and Huland H., Der Onkologe (1996), Vol 2: 218-223 Früherkennung des Prostatakarzinoms. Onkologe
4. Milford Ward A. et al., Ann Clin Biochem (2001), Vol 38: 633-651 Prostate specific antigen: biology, biochemistry and available commercial assays.
5. Price C. P. et al., Ann Clin Biochem (2001), Vol 38: 188-216 Pre-and post-analytical factors that may influence use of serum prostate specific antigen and its isoforms in a screening programme for prostate cancer.
6. Lange P et al., J Urol (1989) Vol 141:873 The value of serum prostate-specific antigen determinations before and after radical prostatectomy,
7. Akdas et al. British J Uro (1997) Vol 79: 920-923 The role of free prostate specific antigen in the diagnosis of prostate cancer
8. Thomas L (2008) Labor und Diagnose, TH-Books Verlagsgesellschaft 1342-1351
9. Gion M et al. (1998) Clin Chem 44: 2462-2470 Percent free prostate specific antigen in assessing the probability of prostate cancer under optimal analytical conditions

Version 2011-09-08~rm