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Product Information

Free Prostate Specific Antigen (f-PSA) ELISA

Catalog Number **SE120056**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Human Prostate Specific Antigen (PSA) is a 33 kDa serine proteinase which, in human serum, is predominantly bound to α_1-antichymotrypsin (PSA-ACT) and α_2-macroglobulin (PSA-AMG). Trace amounts of α_1-antitrypsin and inter-alpha trypsin inhibitor bound to PSA can also be found. Any remaining PSA is in the free form (f-PSA).[1-3] Current methods of screening men for prostate cancer utilize the detection of the major PSA-ACT form. Levels of 4.0 ng/ml or higher are strong indicators of the possibility of prostatic cancer.[4] 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.[5] A potential solution to this problem involves the determination of free PSA levels.[6-17] Preliminary studies have suggested the percentage of free PSA (f-PSA) is lower in patients with prostate cancer than those with benign prostatic hyperplasia.[2] 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.[6]

The Free Prostate Specific Antigen (f-PSA) ELISA kit is used for the quantitative measurement of f-PSA in human serum.

The Free Prostate Specific Antigen (f-PSA) ELISA kit is a solid phase direct sandwich ELISA method. The samples and diluted anti-f-PSA-HRP conjugate are added to the wells coated with MAb to f-PSA. The f-PSA molecules present in the standard solution or sera are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound protein and HRP-conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of f-PSA in the samples. A standard curve is prepared relating color intensity to the concentration of the f-PSA.

Components

Materials Provided	96 Tests
Microwells coated with f-PSA MAb	12 × 8 × 1
f-PSA Standard: 6 vials (ready to use)	0.5 ml
Anti-f-PSA Enzyme Conjugate: 1 bottle (ready to use)	12 ml
TMB Substrate: 1 bottle (ready to use)	12 ml
Stop Solution: 1 bottle (ready to use)	12 ml
20× Wash concentrate: 1 bottle	25 ml

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at 2–8 °C for 5 days. If storage time exceeds 5 days, store frozen at –20 °C for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

20× Wash Buffer Concentrate

Prepare 1× Wash buffer by adding the contents of the bottle (25 ml, 20×) to 475 ml of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, controls, and serum samples be run in duplicate

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Bring all specimens and kit reagents to room temperature (18–26 °C) and gently mix.

1. Place the desired number of coated strips into the holder
2. Pipette 50 µl of f-PSA standards, control, and sera to selected wells.
3. Add 100 µl of Enzyme Conjugate to all wells.
4. Mix the content of the plate, gently, for 30 seconds.
5. Cover the plate and incubate for 60 minutes at room temperature (18–26 °C).
6. Remove liquid from all wells. Wash wells 3 times with 300 µl of 1× Wash buffer. Blot on absorbent paper towels.
7. Add 100 µl of TMB substrate to all wells.
8. Incubate for 15 minutes at room temperature.
9. Add 50 µl of Stop Solution to all wells. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the Stop Solution.

Results
Calculations
1. Calculate the average absorbance values (A_{450}) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of f-PSA in ng/ml from the standard curve.

Example Of Standard Curve
This standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each laboratory should obtain its own data and standard curve.

f-PSA (ng/ml)	Absorbance (450 nm)
0	0.02
1.0	0.14
2.0	0.26
5.0	0.57
10.0	1.13
20.0	2.22

Expected Values
As discussed in the introduction, the important diagnostic parameter is not the level of free PSA, but rather the ratio of free PSA to total PSA. Percent free-PSA offered the greatest advantage to the total PSA test when the total PSA values were between 3.0 and 10.0 ng/ml.[14] For a given patient sample, different commercial test kits of total PSA and free-PSA may give different values of total PSA and free-PSA. Users should keep this in mind while calculating the percentage. The following information is cited from References 6, 7, 10, 11, 13, 14-17. For total PSA levels between 3.0–4.0 ng/ml, using a 19% cutoff point for percent free-PSA would result in detection of 90% of all cancers.[14] For total PSA levels between 4.1–10.0 ng/ml, the most appropriate cutoff point for free-PSA is 24%. At this cutoff point, 95% of the cancers would be detected.[14] With respect to free PSA levels and prostate volume; the available information is again limited. Catalona et al., were the first to demonstrate the importance of prostate size in selecting the cutoff value for percent free-PSA.[13] In their study, men with prostate cancer and a prostate volume of 400 cc or less had a median free-to-total PSA proportion of 0.092 (9.2%), a value statistically lower than the 0.159 (15.9%) found for patients with prostate cancer and a gland >40.0 cc. Yemoto et al., in a recent study of 200 men, showed no correlation between percent free-PSA and prostate volume.[16] Data from several studies have demonstrated an inverse relationship between percent free-PSA and total PSA. This observation suggests higher PSA levels are more commonly associated with lower percent free-PSA values and these men most frequently have more aggressive or advanced prostate cancers.[2,14,17]

Notes: Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

Product Profile

Correlation

A total of 60 sera were tested by this ELISA kit and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.99	0.79	0.011

Sensitivity

The sensitivity of this kit is estimated to be 0.1 ng/mL.

References

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