AccuDiag™ Free PSA ELISA Kit

REF 4322-16

IVD See External Label 2°C 8°C Σ 96 Tests

Free PSA ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	0-10 ng/mL
Sample	100 µL Serum
Specificity	98.7%
Sensitivity	0.05 ng/mL
Incubation Time	160 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The DAI Free-PSA Enzyme Immunoassay test kit is intended for the quantitative determination of f-PSA in human serum.

SIGNIFICANCE AND SUMMARY

Human Prostate Specific Antigen (PSA) is a 33 kD serine proteinase which, in human serum, is predominantly bound to alpha 1-antichymotrypsin (PSA-ACT) and alpha 2-macroglobulin (PSA-AMG). 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 (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.[1-3] Preliminary studies have suggested that the percentage of free 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]

ASSAY PRINCIPLE

The f-PSA ELISA test is a solid phase two-site immunoassay. An anti-f-PSA monoclonal antibody is coated on the surface of the microtiter wells and another anti-PSA monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The f-PSA molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen- antibody-enzyme complex, the unbound antibody-enzyme tracers are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by a colorimetric reaction. The intensity of the color formed is proportional to the concentration of f-PSA present in the sample.

SPECIMEN COLLECTION AND PREPARATION

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as possible. Avoid grossly hemolytic, lipemic, or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with the test procedures and should be avoided.
3. Specimens should be capped and may be stored up to 48 hours at 2-8°C, prior to assaying. Specimens held for a longer time can be frozen at –20°C.
4. Thawed samples must be mixed prior to testing.

MATERIALS AND COMPONENTS

Materials provided with the test kit

1. Antibody-coated microtiter plate with 96 wells.
2. Sample Diluent, 12 ml.
3. Reference standards containing 0, 0.1, 0.5, 2.0, 5.0, and 10.0 ng/ml f-PSA, liquid (ready to use) or lyophilized form. 1 set.
4. Enzyme Conjugate Reagent, 22 ml.
5. TMB Substrate, 12ml
6. Stop Solution, 12 ml.
7. Wash Buffer Concentrate (50X), 15 ml.

Materials required but not provided

1. Precision pipettes: 0.10, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Vortex mixer or equivalent.
5. Absorbent paper or paper towels.
6. Graph paper.
7. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2.5 OD or greater at 450nm.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-22°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. If reference standards are lyophilized, reconstitute each standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.
3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100µl of sample diluent into each well.
4. Thoroughly mix for 10 seconds. It is very important to have a complete mixing in this step.
5. Incubate at 37°C for 60 minutes
6. Remove the incubation mixture by emptying plate contents into a suitable waste container.
7. Rinse and empty the microtiter wells 5 times with washing buffer (1X).
9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
10. Dispense 200µl of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
11. Incubate at 37°C for 60 minutes.
12. Remove the incubation mixture by emptying plate contents into a suitable waste container.
13. Rinse and empty the microtiter wells 5 times with washing buffer (1X).
14. Strike the wells sharply onto absorbent paper to remove residual water droplets.
15. Dispense 100µl TMB solution into each well. Gently mix for 5 seconds.
16. Incubate at room temperature for 20 minutes in the dark.
17. Stop the reaction by adding 100µl of stop solution to each well.
18. Gently mix for 30 seconds to make sure that the blue color changes completely to yellow.
19. Using a microtiter plate reader, read the optical density at 450nm within 15 minutes.

Important Notes:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that if manual pipetting is used, no more than 32 wells be used for each assay run, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.

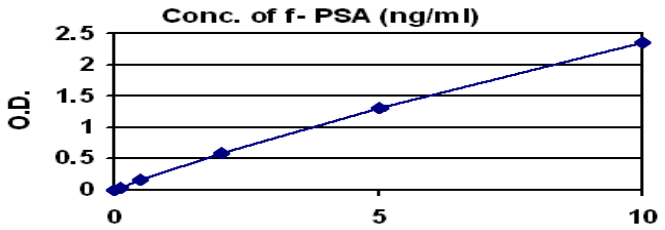
RESULTS

Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls, and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on graph paper. The absorbance values are placed on the vertical, or Y-axis, and concentrations on the horizontal, or X-axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of f-PSA in ng/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of typical standard run with optical density reading at 450nm shown in the Y-axis against f-PSA concentrations shown in the X-axis.
This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own standard curve and data.

f-PSA (ng/ml)	Absorbance (450nm)
0	0.002
0.1	0.065
0.5	0.262
2.0	0.962
5.0	1.976
10.0	2.991



EXPECTED VALUES AND SENSITIVITY

From the literatures, clinical studies have shown that higher tota PSA levels and lower percentages of free PSA are associated with higher risks of prostate cancer. In one study, 52 individuals with benign prostate hyperplasia (BPH) and 77 individuals with prostate cancer were analyzed. The f-PSA/t- PSA ratio were summarized in the table below.

	f-PSA/t-PSA Ratio			
	Median	Min	Max	Mean (95% confidence interval)
BPH	0.18	0.04	0.42	0.19 (0.17-0.21)
Prostate Cancer	0.09	0.02	0.53	0.12 (0.10-0.14)

The relationship between f-PSA/t-PSA ratio and risk of prostate cancer is also age related. When total PSA is in the range of 4.0-10.0 ng/mL, a f-PSA/t-PSA ratio ≤ 0.10 indicates 49% to 65% risk of prostate cancer depending on age; a f-PSA/t-PSA ratio > 0.25indicates a 9% to 16% risk of prostate cancer, depending on age.

	Percent probability of prostate cancer		
f-PSA/t-PSA ratio	50-59 years	60-69 years	≥ 70 years
≤ 0.10	49.2	57.5	64.5
0.11-0.18	26.9	33.9	40.8
0.19-0.25	18.3	23.9	29.7
>0.25	9.1	12.2	15.8

Multiple factors such as population, age, specificity of test method may affect interpretation of f-PSA and t-PSA values. These ranges should be used as guidelines only. Each laboratory should establish its own reference values.

Cross Reactivity

Antigens	Concentration	% Cross-react.
PSA-ACT	500 ng/ml	0.2
AFP	10,000 ng/mL	0
CEA	5,000 ng/mL	0
CA 125	1,000 U/mL	0
CA 15-3	1,000 U/mL	0
CA 19-9	1,000 U/mL	0
∝-HCG	1,000 ng/ml	0
ß-HCG	1,000 ng/mL	0
HCG	50,000 mIU/ml	0

Precision

Intra-Assay			
	Replicates	S.D.	% CV
Level I	20	0.005	13.1
Level II	20	0.011	4.4
Level III	20	0.128	3.2

Linearity

Two patient sera were serially diluted with 0 ng/ml standard. The average recovery was 108.7%.

Sample A			
Dilution	Expected	Observed	% Recovery
undiluted	8.691	8.691	100
2X	4.346	4.455	102.5
4X	2.173	2.290	105.4
8X	1.086	1.258	115.8
16X	0.543	0.617	113.6
32X	0.272	0.294	108.1
64X	0.136	0.147	108.1
			Average Recovery: 107.6%

Sample B			
Dilution	Expected	Observed	% Recovery
undiluted	7.015	7.015	100
2X	3.508	3.516	100.2
4X	1.754	1.834	104.6
8X	0.877	0.970	110.6
16X	0.438	0.509	116.2
32X	0.219	0.249	113.7
64X	0.110	0.136	123.6
			Average Recovery: 109.8%

Recovery

Equal parts of diluted patient sera were mixed to test for interference by unknown materials, such as drugs or hormones, in the assay. Concentrations of Free PSA were determined before (original and added) and after (observed). The average recovery was 99.2%.

Sample 1					
Sample	Orig. Conc	Added	Expected	Observed	% Recovery
A	9.802	0.141	4.972	4.540	91.3
B	9.802	0.281	5.042	4.638	92.0
C	4.699	2.168	3.434	3.342	97.3
D	2.168	1.170	1.669	1.661	99.5
E	0.563	0.281	0.422	0.423	100.2
F	4.699	1.125	2.912	2.727	93.6
G	0.563	0.141	0.352	0.399	113.4
					Average Recovery: 98.2%

Sample 2					
Sample	Orig. Conc	Added	Expected	Observed	% Recovery
A	6.825	0.098	3.462	3.301	95.3
B	6.825	0.195	3.510	3.156	89.9
C	3.342	1.563	2.453	2.503	102.0
D	1.706	0.781	1.244	1.205	96.9
E	0.391	0.195	0.293	0.327	111.6
F	3.342	0.781	2.062	1.964	95.2
G	0.391	0.098	0.245	0.271	110.6
					Average Recovery: 100.2%

LIMITATIONS OF THE PROCEDURE

There are some limitations of the assay:

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit
2. (One year from the date of manufacture). Refer to the package label for the expiration date. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
2. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

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MANUFACTURER AND BRAND DETAILS

ISO 13485-2016

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