Free PSA Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAFPS1	Free PSA Elisa	96 Tests

Intended Use:

The Free PSA Elisa kit is intended for the quantitative determination of Free PSA in human serum.

Summary and Principle:

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). 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. 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. A potential solution to this problem involves the determination of free PSA levels. Preliminary studies have suggested that the percentage of free PSA is lower in patients with prostate cancer than those with benign prostatic hyperplasia. 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.

The f-PSA EIA test is a solid phase two-site immunoassay. An anti-PSA monoclonal antibody is coated on the surface of the microtiter wells and another anti-PSA monoclonal antibody labelled with horseradish peroxidase is used as the tracer. Any f-PSA molecules present in the standard solution or serum are "sandwiched" between the two antibodies and immobilised on the plate.

Following the formation of the antibody-antigen-antibody-enzyme complex, 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 colour formed is proportional to the concentration of f-PSA present in the sample.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with Anti-Free PSA antibody. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Stable until expiry date using the above storage conditions.
FPSA Calibrators	6x1ml	Free PSA reference standards set, containing 0, 0.1, 0.5, 2.0, 5.0 and 10.0 ng/ml liquid stable. Store at 2-8°C. Once opened the Calibrators are stable until expiry date at storage temperature.
Sample Diluent	1x12ml	Buffer based solution for dilution of samples. Store at 2-8°C. Once opened stable until expiry date.
Enzyme Conjugate Reagent	1x22ml	Once opened the material is stable until expiry date when stored tightly capped and without contamination at 2-8°C.
Wash Buffer Concentrate (50X)	1x15ml	PBS-Tween Wash solution. 50X concentrate. Once diluted the wash buffer is stable for 2 months when stored at 15 – 25°C.
TMB Substrate	1x12ml	TMB and Urea Peroxide Solution. Store at 2-8°C. Once opened stable until expiry date.
Stop Solution	1x6ml	Once opened the material is stable until expiry date when stored tightly capped and without contamination at 2-8°C.

IFU and plate covers.

Important Note: All kit components and serum samples should be allowed to equilibrate to room temperature before use.

Materials required but not provided:

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

Precautions and warnings:

1. For *In Vitro* Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapour. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered **POTENTIALLY BIOHAZARDOUS MATERIALS** and handled accordingly.
4. The human serum controls are **POTENTIALLY BIOHAZARDOUS MATERIALS**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centres for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Blood borne Pathogens.
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach a room temperature before starting the assay.** Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of an accident or if you feel unwell, seek medical advice immediately.
8. The TMB Peroxidase Substrate is HARMFUL. Irritating to eyes, respiratory system and skin.
9. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
10. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
11. Reagents from other sources or manufacturers should not be used.
12. TMB Peroxidase Substrate Solution should be colourless, very pale yellow, very pale green or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change colour prematurely. Do not use the TMB if it is noticeably blue in colour. To help reduce the possibility of contamination, refer to Test Procedure, Substrate Incubation section to determine the amount of TMB to be used and dispense into a secondary container only what is needed to properly perform the assay.
13. Dilution or adulteration of these reagents may generate erroneous results.
14. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
15. Avoid microbial contamination of reagents. Incorrect results may occur.
16. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
17. Avoid splashing or generating aerosols.
18. Do not expose reagents to strong light during storage or incubation.
19. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
20. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
21. Do not allow the conjugates to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
22. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odours from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

Specimen Collection:

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 haemolytic, 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. Thawed samples must be mixed prior to testing.

Storage and Stability:

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 (One Year from the date of manufacture). Refer to the package label for the expiration date
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. 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.

Procedure:

STEP 1

Preparation:

- 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.
- If reference standards are lyophilised, reconstitute each standard with 0.5ml of distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.
- Dilute 1 volume of Wash Buffer Concentrate (50X) with 49 volumes of distilled water. For example, dilute 15ml of Was Buffer (50X) into distilled water to prepare 750 ml of Washing Buffer (1x). Mix well before use.

STEP 2

Addition of Standards and Specimen: Secure the desired number of coated wells in the holder. Dispense 100 µl of standards, specimens, and controls into appropriate wells.

STEP 3

Addition of Sample Diluent: Add 100 µl of the Sample Diluent into each well. Mix thoroughly for 10 seconds. It is very important to have a complete mixing in this step.

STEP 4

Incubation: Incubate for 60 minutes at 37°C.

STEP 5

Washing: Remove the incubation mixture by emptying plate contents into a suitable waste container. Rinse and empty the microwell plate 5 times with washing buffer (1X). Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

STEP 6

Addition of Enzyme Conjugate: Add 200 µl of Enzyme Conjugate to each well. Gently mix the mixture for 5 seconds.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at 37°C.

STEP 8

Washing: Remove the incubation mixture by emptying plate contents into a suitable waste container. Rinse and empty the microwell plate 5 times with washing buffer (1X). Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

STEP 9

Addition of TMB Substrate: Dispense 100 µl of TMB Substrate into individual well. Gently mix for 5 seconds.

STEP 10

Incubation: Incubate at room temperature for 20 minutes in the dark.

STEP 11

Stopping the Reaction: Add 100 µl of the Stop solution into each well. Gently mix for 30 seconds to make sure that the blue colour changes completely to yellow. Using a microwell 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 3 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.

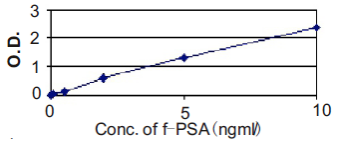
Calculation of Results:

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 free 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 free 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 their own standard curve and data.

Free PSA (ng/ml)	Absorbance (450nm)
0.0	0.006
0.1	0.032
0.5	0.155
2.0	0.597
5.0	1.302
10.0	2.361



Precision:

1. Intra-Assay:

Concentration	Replicates	Mean	S.D.	% CV
Level I	20	0.038	0.005	13.1
Level II	20	0.250	0.011	4.4
Level III	20	4.00	0.12	3.2

2. Inter-Assay:

Concentration	Replicates	Mean	S.D.	% CV
Level I	20	0.041	0.006	15.8
Level II	20	0.280	0.019	6.8
Level III	20	4.10	0.209	5.1

Linearity:

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

Sample A			
Dilution	**Expected**	**Observed**	**% Recov.**
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**	**% Recov.**
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**	**% Recov.**
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**	**% Recov.**
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%					

Sensitivity:

The minimum detectable concentration of free PSA in this assay is estimated to be 0.05 ng/ml.

Cross-reactivity:

The following human materials were tested for cross-reactivity of the assay:

Antigens	Concentration	% Cross-react.
PSA-ACT	500 ng/ml	0.2
AFP	10000 ng/ml	0
CEA	5000 ng/ml	0
CA 125	1000 U/ml	0
CA 15-3	1000 U/ml	0
CA19-9	1000 U/ml	0
∝-HCG	1000 ng/ml	0
ß -HCG	1000 ng/ml	0
HCG	50000 mIU/ml	0

Hook Effect:

No hook effect was observed in this assay.

References

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REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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