



Prostate Specific Antigen (PSA) MICRO-ELISA Test Kit

Prod. No.: T179
Pkg. Size: 96 Tests

CAUTION: The concentrations of Total PSA in a given specimen determined with assays from different manufacturers can vary due to differences in assay method and reagent specificity. The results reported by the laboratory to the physician must include identity of the PSA assay used. The total PSA values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining PSA levels serially is changed, additional sequential testing should be carried out to confirm baseline values.

Description

Enzyme Immunoassay for the Quantitative Determination of Prostate Specific Antigen (PSA) in Human Serum.

Summary and Explanation of the Test

A prostate specific protein, gamma-seminoprotein (γ SM) was described by Hara, *et al.*, in 1971¹ and in 1978 Sensabaugh characterized the prostate specific nature of "Seminal Plasma Protein p30".² This protein was later described (1979) by Wang, *et al.*, as PSA (prostate specific antigen).³ The identity of PSA, gamma-seminoprotein (γ SM) and p30 was established in 1990.^{4,5} This protein is a glycoprotein monomer with a 33-34,000 molecular weight and significant protease activity.^{3,6} This prostrate specific antigen is secreted by normal prostrate epithelium as well as prostate cancer cells.^{7,8}

The clinical utility of blood PSA measurements was confirmed by findings that established an exclusive association between blood PSA with prostate tissue.⁹ In healthy male subjects the serum PSA value is less than 4 ng/ml (99%). An increase in PSA value to above 4 ng/ml is found in males with benign prostatic hypertrophy (BPH) as well in patients malignant prostate disease. Clinical studies have demonstrated the importance of PSA testing in patient with metastatic or persistent disease following treatment of prostate cancer.^{10,11}

Results from PSA determinations are **not** absolute evidence for the presence or absence of malignant disease. Studies indicate that up to 60% of patient with cancerous prostate disease (Stage A) will have PSA values of less than 4 ng/ml, and that up to 30% of males with BPH will have PSA values greater than 4 ng/ml.¹²⁻¹⁶ Additionally, specimens from patients undergoing prostrate manipulation procedures or hormonal therapy may give misleading PSA results.^{17,18} PSA results must be interpreted in the light of other clinically acceptable tests and procedures.

Prostate Specific Antigen (PSA) 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- α -trypsin inhibitor bound to PSA can also be found. Any remaining PSA is in the free form (**f-PSA**).¹⁹⁻²¹ Current methods of screening men for prostate cancer utilize the detection of the major PSA-ACT form. Levels of 4.1 ng/ml or higher are strong indicators of the possibility of prostatic cancer.²² 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.²³

Principle of the Procedure

The MICRO-ELISA PSA test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes two unique antibodies (mouse monoclonal) directed against distinct antigenic determinants on the PSA molecule.

Specifically, plastic wells are coated with anti-PSA (mouse monoclonal). With the addition of a calibrator, test sample or appropriate control containing PSA, immune complexes are formed between free PSA in the sample and the solid phase anti-PSA. Anti-PSA (mouse monoclonal) enzyme-labeled with horseradish peroxidase is added to each well. During an incubation period (45 minutes), the PSA molecule is sandwiched between the solid phase and enzyme-labeled antibodies. The wells are then decanted and washed to remove unbound labeled antibody. An enzyme substrate-chromogen (hydrogen peroxide, H_2O_2 , and tetramethylbenzidine, **TMB**) is added to the well and incubated for 15 minutes at room temperature, resulting in the development of a blue color. The addition of 1.0 N H_2SO_4 stops the reaction and converts the color to yellow and increases the absorbance by a factor of approximately 3. The intensity of the yellow color is directly proportional to the concentration of PSA in the sample. A calibration curve constructed from the 450 nm absorbance of the 0, 5, 15, 40, 75 and 100 ng/ml PSA calibrators will allow calculation of sample values.

Reagents

Components in Each 96-Test

MICRO-ELISA PSA Diagnostic Kit

- 96 wells, PSA ANTIBODY COATED WELLS: Coated with anti-PSA (mouse monoclonal); contained in a pack with silica gel desiccant.
- 1 bottle, 22 ml, PSA ENZYME ANTIBODY CONJUGATE: anti-PSA (mouse monoclonal) labeled with horseradish peroxidase in buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.



- 1 bottle, 12 ml, **SUBSTRATE-CHROMOGEN** Buffered hydrogen peroxide and 3,3',5,5'- tetramethylbenzidine (TMB) solution.
- 1 bottle, 12 ml, **STOP SOLUTION** 1 N H₂SO₄.
- 1 vial, 4 ml, 0 ng/ml, PSA **CALIBRATOR** Human Serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as a preservatives.
- 5 vial, 1 ml, PSA **CALIBRATORS** Bovine Serum with added human PSA; contain 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. **5, 15, 40, 75 and 100 ng/ml.**
- 2 vial, 1 ml, PSA **CONTROLS** Bovine Serum with added human PSA; contain 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. **NORMAL AND ELEVATED.**
- 1 bottle, 60 ml, **WASH BUFFER CONCENTRATE (20X)**: Buffered detergent solution, contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. Dilute bottle to 1200 ml with deionized water.

Additional Materials Required

Precision pipettes: 0.050, 0.10 and 0.20 ml.
Disposable pipette tips.
Distilled water.

Storage and Stability

Store unopened kits at 2°-8°C. The following components may be stored at **ambient temperature**; **WELLS, WASH BUFFER, SUBSTRATE-CHROMOGEN and STOP SOLUTION**. Expiration date printed on the kit indicates limits of stability.

The PSA **ANTIBODY COATED WELLS** are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells can be stored at 2° - 30°C.

Chemical or Physical Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent contamination or deterioration.

Instruments

Performance of the **PSA** test requires use of a precision microtiter plate reader at a wavelength of 450 ± 20 nm.

Specimen Collection and Preparation

Serum samples are used in the **PSA** Diagnostic Kit procedure. No special preparation of the patient is necessary; fasting is not required. Repeated freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

STORAGE: Specimens may be stored in a tightly sealed tube at 2°-8°C for 48 hours. If the serum is not assayed within 2 days, store frozen (-20°C) in a tightly sealed tube for up to 3 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.

Micro-ELISA PSA Procedure

Reagent Preparation

Dilute bottle of **WASH BUFFER CONCENTRATE (20X)** solution to 1200 ml with deionized water. Diluted wash solution will be stable until the expiration date stamped on the kit.

Preliminary Comments and Precautions

Patient sample may contain pathogens: treat all samples as potentially infectious.

Reagents contain thimerosal; avoid contact with skin.

Avoid contact with **SUBSTRATE-CHROMOGEN** (tetramethylbenzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).

CAUTION: Source material used to prepare Calibrators was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. **HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS.** Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

Procedural Notes

- All test kit components used in the assay must be of the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (18° - 30°C) before testing begins.
- All calibrators, controls, and samples should be tested in duplicate simultaneously. The test samples and controls must be well mixed before use.
- A separate disposable tip should be used for each sample to avoid cross-contamination. All pipetting steps should be performed with the utmost care and accuracy. Avoid contaminating the reagent pipette tip with the serum sample.
- The duration of the incubation times must be the same for all wells within a run.
- Run size should be limited to the number of samples that can be added to antibody coated wells within 5 minutes.
- Samples should be pipetted to the bottom of the antibody-coated wells.
- If microtiter reader is not capable of reading absorbances greater than 2.5, the color should be read after a shorter incubation time with the SUBSTRATE/CHROMOGEN, i.e., 10 minutes.**

Assay Procedure

- Place sufficient **COATED WELLS** in a holder to run 0.0, 5, 15, 40, 75 and 100 ng PSA/ml **CALIBRATORS**, Quality Control Sera and patient samples in duplicate. **Limit run size** to the number of samples that can be pipetted in **5 minutes**.
- Pipet **50 µl** of standards, specimens, and controls into appropriate wells.



3. Dispense **200 µl** of Enzyme Conjugate Reagent into each well. Gently mix for 5 seconds.
4. Incubate at room temperature (18°-30°C.) for **45 minutes ± 5 minutes**.
5. Decant or aspirate and discard liquid contents of all wells. **SLAP** the inverted wells on a clean piece of absorbent paper. Remove **ALL OF THE LIQUID** from the wells.
6. Fill each well with diluted WASH BUFFER. **Fill the wells to overflowing**. Decant or aspirate liquid contents of all wells. **SLAP** the inverted wells on a **fresh** clean piece of absorbent paper. Remove **ALL OF THE LIQUID** from the wells.

WARNING: WASHING THE WELLS IS OF CRITICAL IMPORTANCE. Fill the wells to overflowing, you CANNOT cause any carryover between wells. You CANNOT over wash the wells. Completely decant or aspirate all of the liquid out of the wells. SLAP the inverted wells on a FRESH clean piece of absorbent paper AFTER EACH WASH. YOU CANNOT SLAP TOO HARD, REMOVE ALL OF THE LIQUID FROM THE WELLS.

7. Repeat step 6 three more times (for a total of 4 washes).
8. Fill each well with deionized water. **Fill the wells to overflowing**. Decant or aspirate liquid contents of all wells. **SLAP** the inverted wells on a **fresh** clean piece of absorbent paper. Remove **ALL OF THE LIQUID** from the wells.
9. Pipet or dispense **100 µl** (0.1 ml) of **SUBSTRATE-CHROMOGEN** solution into each well.
10. Mix thoroughly and incubate 15 minutes at room temperature (18-30°C).
11. Pipet or dispense **100 µl** (0.1 ml) of 1 N H₂SO₄ into each well and mix thoroughly.
12. Read the absorbance of each well at 450 ± 10 nm against water.

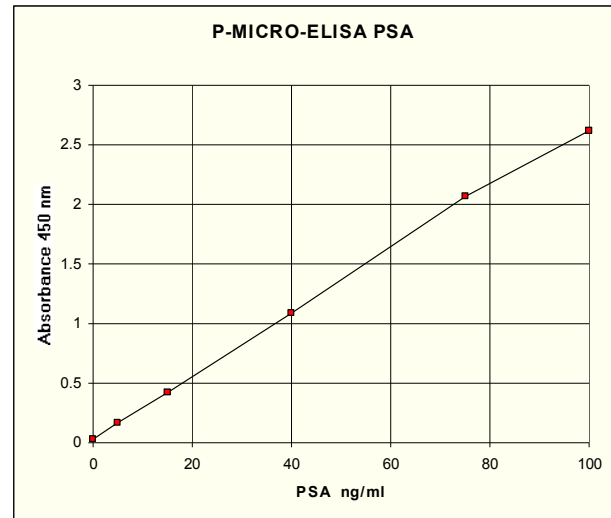
Calculation of Results

1. Calculate the mean value for each duplicate sample absorbance at 450 nm. Values for duplicate absorbances should be within 10% (or 0.02 absorbance units for absorbances less than 0.2).
2. Construct a standard curve by plotting the mean absorbance obtained for each PSA CALIBRATOR on the vertical (Y) axis versus the corresponding PSA concentration on the horizontal (X) axis, using rectilinear graph paper.
3. Connect the points with straight-line segments.
4. Using the mean absorbance for each sample, read the corresponding PSA concentration in ng/ml from the curve. Multiply the value by the dilution factor if required.

EXAMPLE DATA
DO NOT USE IN PLACE OF CUREVE
DETERMINED AT THE TIME OF ASSAY.

Specimen I.D.	A ₄₅₀	Mean A ₄₅₀	PSA(ng/ml)
CALIBRATOR			
0 ng/ml	0.026	0.026	0.026
5 ng/ml	0.173	0.160	0.167
15 ng/ml	0.425	0.417	0.421
40 ng/ml	1.044	1.128	1.086
75 ng/ml	2.071	2.058	2.065
100 ng/ml	2.624	2.609	2.617

SAMPLES				
# 1	0.087	0.086	0.087	2.15
# 2	0.384	0.391	0.388	13.7
# 3	0.808	0.818	0.813	29.7



Quality Control

Good laboratory practice requires that quality control specimen be run with each calibration curve to check the assay performance. Controls containing azide can not be used. Pooled human serum or commercially available control sera without azide are also suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Read the absorbance of the test solutions against a water blank. If the absorbance of the 0 ng/ml CALIBRATOR exceeds 0.100 it is an indication of careless washing and the assay must be repeated.

Limitations of the Procedure

1. 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.
2. All reagents should be allowed to come to room temperature prior to beginning the assay.
3. When it is necessary to measure levels of PSA greater than the 100 ng/ml CALIBRATOR, the sample should be diluted with DILUENT and re-assayed.



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- The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbances. The use of tap water for washing could result in a higher background absorbance.
- FINAL REACTION STABILITY:** Spectrophotometric measurement should be made within thirty minutes after the addition of the STOP solution.
- 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.
- Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies.³¹⁻³² Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). These samples should not be assayed with the MICRO-ELISA PSA test as erroneous results may be obtained.^{28²⁷⁻³⁰} These conditions should be ruled out prior to clinical evaluation of test results.
- Samples with elevated levels of PSA (up to 100,000 ng/ml) will always assay as >100 ng/ml when tested, and will not result in a "high dose hook effect". When it is necessary to measure levels of PSA greater than the 100 ng/ml CALIBRATOR, the sample should be diluted with the 0 ng/ml CALIBRATOR and re-assayed.

Expected Values

PSA values were measured in serum samples from 392 apparently normal individuals and the following results were obtained:

	PSA (ng/ml)
ADULT MALE	0.0 - 4.0

Performance Characteristics of the Test

Assay Sensitivity

The sensitivity of this assay is defined as the smallest single value that can be distinguished from the zero calibrator. This value was calculated from the mean + two standard deviations for twenty-two replicates at the zero concentration. The calculated sensitivity is < 0.2 ng/ml.

Cross Reactivity

Antigens	Concentration	% Cross-reactivity
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
α-hCG	1,000 ng/ml	0
β-hCG	1,000 ng/ml	0
i-hCG	50,000 mIU/ml	0

Assay Reproducibility

Intra-assay reproducibility was determined by measurement of 20 replicates of three serum pools in a single run.

	Mean PSA (ng/ml)	SD	%CV
Serum A	1.67	0.13	7.52
Serum B	5.87	0.29	4.92
Serum C	67.19	3.34	4.97

The interassay reproducibility was determined by duplicate measurement of three serum pools in nine separate runs.

	Mean PSA (ng/ml)	SD	%CV
Serum A	1.66	0.15	9.23
Serum B	6.03	0.40	6.57
Serum C	48.76	2.07	4.24

Assay Linearity

A study was performed diluting a serum sample containing an elevated level of PSA with the 0 ng/ml CALIBRATOR to determine the linearity of the MICRO-ELISA PSA.

Dilution Factor	Expected Value (ng/ml)	Observed Value (ng/ml)	% of Expected Value
undiluted	-	74.77	-
4:5	59.82	63.90	107 %
3:5	44.86	48.52	108 %
2:5	29.21	30.98	104 %
1:5	14.95	14.40	96 %
1:10	7.48	7.47	100 %
1:20	3.74	4.09	110 %

Assay Recovery

Five aliquots of human sera with a PSA concentration of 9.50 ng/ml were spiked with 13.25, 24.29, 41.64, 54.65 and 64.77 ng/ml of PSA. The samples were assayed in duplicate.

Added PSA ng/ml	PSA ng/ml		% Recovery
	Expected Value	Measured Value	
0	-	9.50	
13.25	22.75	23.34	103 %
24.29	33.79	32.73	100 %
41.64	51.14	50.80	99 %
54.65	64.15	66.26	103 %
64.77	74.27	79.48	107 %

Assay Specificity

Specificity of this test system was determined by assaying 775 male serum samples which had been assayed for PSA by either the Abbott (IMX) or Hybritech (ELISA) PSA tests.

PRECISA AGREEMENT WITH OTHER (ABBOTT OR HYBRITECH) PSA

	VALUE	
	< 4.0 ng/ml	> 4.0 ng/ml
# OTHER PSA	392	383
PRECISA AGREEMENT	370	374
% AGREEMENT	94.4%	97.6%
PRECISA DISAGREEMENT	22	9
% DISAGREEMENT	5.6%	2.3%
OVERALL AGREEMENT 744 OUT OF 755		(96.0%)



OTHER PSA VALUE	PRECISA VALUE	DIFFERENCE (ng/ml)
2.9	4.0	1.1 high
3.1	4.1	1.0 high
3.2	4.2	1.0 high
3.2	4.6	1.4 high
3.3	4.0	0.7 high
3.3	4.3	1.0 high
3.4	4.1	0.7 high
3.4	4.9	1.5 high
3.7	4.4	0.7 high
3.7	4.6	0.9 high
3.8	5.0	1.2 high
3.8	4.9	0.9 high
3.8	4.3	0.5 high
3.8	4.0	0.2 high
3.9	5.1	1.2 high
3.9	4.4	0.5 high
3.9	4.4	0.5 high
3.9	5.4	1.5 high
4.0	4.8	0.8 high
4.0	4.9	0.9 high
4.0	4.2	0.2 high
4.0	4.1	0.1 high
4.1	3.7	0.4 low
4.1	3.6	0.5 low
4.2	3.9	0.3 low
4.2	3.8	0.4 low
4.2	3.5	0.7 low
4.2	3.6	0.6 low
4.7	3.9	0.8 low
5.6	3.6	2.0 low

Additionally the specificity of this test system was determined by assaying 50 female serum samples. All of the female samples assayed for PSA gave results < 0.1 ng/ml.

Comparison to Other PSA Tests

Correlation studies on a group of 676 serum samples with a range of values from <0.1 - 99 ng/ml, were performed using the results from the MICRO-ELISA PSA Test and the Abbott IMX PSA test. The correlation coefficient of the test results was 0.976. Another correlation study on a group of 94 serum samples with a range of values from <0.1 - 99 ng/ml, was performed using the MICRO-ELISA PSA Test and the Hybritech Tandem-E PSA test. The correlation coefficient of the test results was 0.989.

	Slope	Y-Intercept	Correlation Coefficient
OVERALL n= 770	1.095	-0.02	0.984
ABBOTT (IMX) n= 676	1.163	-0.24	0.976
HYBRITECH TANDEM-E n= 94	0.783	0.67	0.992

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