IMMULITE®

Free PSA

For use on the IMMULITE®
and IMMULITE® 1000 systems

37

DPC®

IMMULITE®/IMMULITE® 1000 Free PSA

English

Intended Use:

For *in vitro* diagnostic use with the IMMULITE®/IMMULITE® 1000 Analyzer for the quantitative measurement of free prostate-specific antigen (PSA) not bound to α1-antichymotrypsin or other binding proteins (uncomplexed) in human serum (including serum collected in serum glass, serum plastic, and serum gel separator tubes). Measurement of Free PSA is used in conjunction with IMMULITE®/IMMULITE® 1000 Total PSA to determine a ratio of Free PSA to Total PSA (percent Free PSA). The percent Free PSA is used as an aid in discriminating prostate cancer from benign disease in men 50 years or older with IMMULITE®/IMMULITE® 1000 Total PSA values between 4 and 10 ng/mL and digital rectal exam (DRE) findings not suspicious of cancer. Prostate biopsy is required for the diagnosis of prostate cancer.

Catalog Number: **LKPF1** (100 tests), **LKPF5** (500 tests)

Test Code: **fPS** Color: **Light Gray**

> The concentration of free PSA in a given specimen determined with different assays can vary due to the differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Free and total PSA values should be obtained using assays from the same manufacturer.

Summary and Explanation

Since the inception of prostate-specific antigen (PSA) measurements by immunoassay, it has been observed that several distinct immunoreactive species could be isolated from the serum of prostate cancer (PCa) patients after molecular sieve chromatography.[1] PSA has been shown to form stable complexes with two of the major extracellular protease inhibitors in blood, α_1-antichymotrypsin (ACT) and α_2-macroglobulin (AMG).[2] In prostate cancer patients, PSA complexed with ACT (PSA-ACT) is typically the major form in circulation: for about 50% of these patients, PSA-ACT accounts for 85% of the total PSA present. Some 12–15% of prostate cancer patients, on the other hand, present with a significantly higher proportion of free (uncomplexed) PSA.[3]

Biochemical studies of PSA isolated from seminal plasma show that approximately 35% is free, being enzymatically inactive and unreactive with protease inhibitors.[4] According to current hypotheses, this form of the molecule represents *either* an inactive zymogen of PSA *or* a nicked or enzymatically inactive form.

Complex formation with ACT results in exposure of a limited number of the antigenic epitopes of PSA, whereas complex formation with AMG encapsulates the antigenic epitopes of PSA. Differences in recognition of these multiple forms of PSA by reagent antibodies have contributed to discrepancies between commercial PSA assays.[2]

Immunometric assays have now been designed to selectively characterize all the molecular forms of PSA in circulation: some detect just PSA-ACT, others just free PSA, and some are considered to be assays for total PSA, detecting PSA epitopes available both on free (uncomplexed) PSA and on PSA complexed to serine protease inhibitors.

Using assays such as these, free PSA was found to comprise a significantly ($p < 0.0001$) smaller fraction of the total PSA concentration in patients with untreated PCa than in patients with benign prostatic hypertrophy (BPH).[3]

A substantial body of literature indicates the benefit of combining free and total PSA in the form of a ratio, facilitating discrimination between PCa and BPH. These determinations must be made using assays developed by the same manufacturer.[5,6] The free-to-total ratio (% free PSA) is typically expressed as a percent: 100 × free PSA / total PSA, with both measured in ng/mL. The % free PSA is, on average, lower in PCa than in BPH and has been commonly used as an aid in PCa diagnosis when the total PSA concentration falls in the "gray zone," that is, between 4 and 10 ng/mL.[7-10] While a lower cutoff might result in fewer false-positive results, a higher cutoff is less likely to miss actual instances of PCa. The % free PSA has also been investigated in the wider total PSA concentration range of 2.0 to 20 ng/mL.[6,8,9,11]

The optimal % free PSA cutoff depends on other factors as well, one of which is the prostate and/or transition zone volume measured by transrectal ultrasound (TRUS).[12-13] Age is another factor: while both free and total PSA rise with advancing age, the % free PSA decreases.[14] More complex approaches employing artificial neural networks (ANNs) take these parameters and others into account, e.g., the results of a digital rectal exam (DRE). Using an ANN can significantly increase discrimination between BPH and PCa.[15]

Principle of the Procedure

IMMULITE/IMMULITE 1000 Free PSA is a solid-phase, sequential chemiluminescent immunometric assay.

The solid phase (bead) is coated with monoclonal murine anti-PSA antibody specific for free (uncomplexed) PSA. The patient sample is incubated with the bead during the first cycle at which time free PSA in the sample binds to the free PSA-specific monoclonal antibody-coated bead. Unbound serum is then removed by a centrifugal wash. Alkaline phosphatase (bovine calf intestine) conjugated to a goat anti-PSA polyclonal antibody in the reagent is introduced in the second cycle and binds to free PSA on the bead to form an antibody-sandwich complex. Unbound enzyme conjugate is then removed by a centrifugal wash. Finally, chemiluminescent substrate is added to the bead and signal is generated in proportion to the bound enzyme.

Incubation Cycles: 2 × 30 minutes.

Specimen Collection

The use of an ultracentrifuge is recommended to clear lipemic samples.

Hemolyzed or grossly contaminated samples may give erroneous results.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Blood collection tubes from different manufacturers may yield differing values, depending on materials and additives, including gel or physical barriers, clot activators and/or anticoagulants. IMMULITE/IMMULITE 1000 Free PSA has not been tested with all possible variations of tube types. Consult the section on Alternate Sample Types for details on tubes that have been tested.

Volume Required: 25 µL serum. (Sample cup must contain at least 100 µL more than the total volume required.)

Storage: 24 hours at 2–8°C or for longer at –20°C.[16,17]

Warnings and Precautions

For *in vitro* diagnostic use.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water.

Materials Supplied

Components are a matched set. The barcode labels are needed for the assay.

Free PSA Test Units (LPF1)
Each barcode-labeled unit contains one bead coated with monoclonal murine anti-PSA antibody specific for free (uncomplexed) PSA. Stable at 2–8°C until expiration date.
LKPF1: 100 units. **LKPF5:** 500 units.

Allow the Test Unit bags to come to room temperature before opening. Open by cutting along the top edge, leaving the ziplock ridge intact. Reseal the bags to protect from moisture.

Free PSA Reagent Wedges (LPFA, LPFB)
With barcodes. **LPFA:** 7.5 mL of a protein buffer matrix, with preservative. **LPFB:** 7.5 mL alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat anti-PSA antibody specific for PSA in a buffer containing human serum, with preservative. Store capped and refrigerated: stable at 2–8°C until expiration date. Recommended usage is within 30 days after opening when stored as indicated.
LKPF1: 1 set. **LKPF5:** 5 sets.

Free PSA Adjustors (LPFL, LPFH)
Two vials (Low and High) of lyophilized free PSA in a buffer solution, with preservative. Reconstitute each vial with **3.0 mL** distilled or deionized water. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved. Stable at 2–8°C for 30 days after reconstitution, or for 6 months (aliquotted) at –20°C.
LKPF1: 1 set. **LKPF5:** 2 sets.

Kit Components Supplied Separately

Free PSA Sample Diluent (LPFZ)
For the manual dilution of patient samples. 25 mL of a PSA-free buffer solution, with preservative. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.

LSUBX: Chemiluminescent Substrate
LPWS2: Probe Wash Module
LKPM: Probe Cleaning Kit
LCHx-y: Sample Cup Holders (barcoded)
LSCP: Sample Cups (disposable)
LSCC: Sample Cup Caps (optional)

TMCO: Tumor Marker Controls (Tri-level, multi-constituent, human serum-based control)

Also Required
Sample transfer pipets, distilled or deionized water, controls.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE or IMMULITE 1000 Operator's Manual.

See the IMMULITE or IMMULITE 1000 Operator's Manual for: preparation, setup, dilutions, adjustment, assay and quality control procedures.

Visually inspect each Test Unit for the presence of a bead before loading it onto the system.

Note that both Reagent Wedges A and B must be loaded on the carousel to run this assay.

Recommended Adjustment Interval: 2 weeks.

Quality Control Samples: Use controls or sample pools with at least two levels (low and high) of free PSA.

Expected Values

A reference range study for IMMULITE/IMMULITE 1000 Free PSA was performed at one U.S. clinical site using serum samples from 119 apparently healthy (no underlying diseases and absence of infection/fever for the previous 2–4 weeks to blood draw) male volunteers ranging in age from 50–77 years. The mean age was 61 years (SD 6.6) and the median was 60 years.

The median and 95th percentile for IMMULITE/IMMULITE 1000 Free PSA results are tabulated below. The percentile was determined nonparametrically.

Group	Median	95%ile	Units	n
Males	0.22	0.62	ng/mL	119

Consider these limits as *guidelines* only. Each laboratory should establish its own reference ranges.

Limitations

IMMULITE/IMMULITE 1000 Free and Total PSA assays should be used to calculate the % free PSA. Percent free PSA values should not be interpreted as absolute evidence for the presence or absence of malignant disease.[18]

Prediction of malignant prostatic disease should be based on a complete clinical evaluation of the patient, which may also include serial serum free and total PSA determinations.

The results of the IMMULITE/IMMULITE 1000 Free PSA assay are only valid when total PSA is in the range of 4 to 10 ng/mL.

Samples should be obtained before biopsy, prostatectomy or prostatic massage.[19]

The assay was studied in a population of 354 patients where 81% were 50–69 years of age and 59% had positive biopsies for prostate cancer. The prevalence of prostate cancer in the patients accrued for the clinical study is higher than that which occurs in the general population. Clinical performance results may not be the same in a population with lower prevalence of prostate cancer.

A sample containing approximately 1 ng/mL Free PSA was spiked with approximately 9 ng/mL complexed PSA (PSA complexed to alpha-1 antichymotrypsin). The difference in the IMMULITE family Free PSA assay result before spiking complexed PSA (1.06 ng/mL) and after spiking complexed PSA (1.14 ng/mL) was 7.5%.

The accuracy of free PSA assays cannot be evaluated by determining the recovery of free PSA spiked into serum samples, because the formation of complexes between free PSA and the PSA-binding proteins present in normal sera (α_1-antichymotrypsin and α_2-macroglobulin) will generally cause erroneously low recoveries.

Some individuals have antibodies to mouse protein that can cause interference in immunoassays that employ antibodies derived from mice. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy, in particular, may contain human anti-mouse antibodies (HAMA). These specimens may show erroneous results in such assays.[20-22] Therefore, IMMULITE Free PSA results should be used only in conjunction with results from some other diagnostic procedure and information available from the clinical evaluation of the patient.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Performance Data

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed in ng/mL. (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

Calibration Range: 0.07 to 25.0 ng/mL
(WHO NIBSC 1st IS 96/668)

Analytical Sensitivity: Limit of Blank (highest value expected for a sample with no analyte; calculated as the value lying two standard deviations above that of the lowest calibrator):
0.02 ng/mL

Limit of Detection (lowest detectable concentration; determined in accordance with CLSI EP17-A[24]):
0.07 ng/mL

Functional Sensitivity: (concentration with 20% coefficient of variation (CV) determined in accordance with CLSI EP17-A[24]:
0.07 ng/mL

High-dose Hook Effect: None up to 14,555 ng/mL.

Precision: Samples were assayed in quadruplicate over 20 days, 1 run per day, for a total of 20 runs and 80 replicates. (See "Precision" table.)

Linearity: The assay is linear to 25.0 ng/mL. Samples assayed under various dilutions demonstrated linearity. (See "Linearity" table for representative data.)

Specificity: IMMULITE/IMMULITE 1000 Free PSA is highly specific for prostate-specific antigen, with a particularly low cross-reactivity to other naturally occurring compounds that might be present in patient samples including alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), ferritin, human chorionic gonadotropin (HCG), prostatic acid phosphatase (PAP), and prolactin. (See "Specificity" table.)

Bilirubin: Presence of bilirubin in concentrations up to 200 mg/L has no effect on results within the precision of the assay.

Hemolysis: Presence of red blood cells may interfere in the IMMULITE/IMMULITE 1000 Free PSA assay, causing small elevations in observed values. (See "Hemolysis" table.)

Lipemia: Presence of triglycerides in concentrations up to 3,000 mg/dL has no effect on results within the precision of the assay.

Alternate Sample Type: To assess the effect of alternate sample types, blood was collected from 22 volunteers into serum glass, serum plastic and serum gel separator tubes (SST). Linear regression analysis indicates equivalence among sample/tube types as follows:

(Serum Plastic) = 0.995 (Serum Glass) +
0.0084 ng/mL
r = 0.998

(SST Plastic) = 0.992 (Serum Plastic) +
0.0017 ng/mL
r = 0.996

Means:
4.48 ng/mL (Serum Glass)
4.46 ng/mL (Serum Plastic)
4.44 ng/mL (SST)

Method Comparison: IMMULITE/IMMULITE 1000 Free PSA was compared to IMMULITE 2000 Free PSA on 680 samples enrolled at 4 clinical sites (concentration range 0.05 – 2.80 ng/mL) See Method Comparison graph. Linear regression analysis indicates that free PSA assays are equivalent across IMMULITE/IMMULITE 1000 (IML) and IMMULITE 2000 (IML 2000) instruments:

(IML 2000) = 1.00 (IML) – 0.003 ng/mL
r = 0.98

Means:
0.59 ng/mL (IML)
0.59 ng/mL (IML 2000)

Clinical Performance: A study was undertaken to assess the clinical utility of the IMMULITE/IMMULITE 1000 Free PSA assay for differentiating between benign prostatic conditions and prostate cancer, using percent free PSA determinations in 354 patients from 5 clinical sites with total PSA levels between 4 – 10 ng/mL and negative digital rectal examinations.

Among the 354 patients analyzed, 81% were 50–69 years of age and 59% had positive biopsies for prostate cancer.

Using the biopsy-confirmed diagnosis of prostate cancer (n = 208) versus no prostate cancer (n = 146) as the reference, Receiver Operator Characteristic Curve analysis was used to evaluate the clinical performance of the free/total PSA ratio at different diagnostic cutoffs. The area under the curve was 0.66 (95% CI = 0.60 – 0.72).

The cutoff of 20% yields a sensitivity of 95% (95% CI = 91% – 98%) and a specificity of 16% (95% CI = 10% – 23%).

The cutoff of 16% yields a sensitivity of 85% (95% CI = 79% – 89%) and a specificity of 36% (95% CI = 29% – 45%).

References

1) Chu TM, et al. Circulating antibody to prostate antigen in patients with prostatic cancer. Ann NY Acad Sci 1983;417: 383-9. 2) Zhou AM, et al. Multiple forms of prostate-specific antigen in serum: differences in immunorecognition by monoclonal and polyclonal assays. Clin Chem 1993;39:2483-91. 3) Lilja H, et al. Prostate-specific antigen in serum occurs predominantly in complex with α_1-antichymotrypsin. Clin Chem 1991;37:1618-24. 4) Christensson A, Laurell CB, Lilja H. Enzymatic activity of prostate-specific antigen and its reactions with extrecellular serine protease inhibitors. Eur J Biochem 1990;194(3):755-63. 5) Wolff JM, Stocker G, Borchers H, Haubeck H, Greiling H, Jakse G. Critical aspects related to the interpretation of the free-to-total PSA-ratio. Anticancer Res 1999;19:2633-6. 6) Patel D, White PA, Milford Ward A. A comparison of six commercial assays for total and free prostate specific antigen (PSA): the predictive value of the ratio of free to total PSA. BJU Int 2000;85:686-9. 7) Correale M, Pagliarulo A, Donatuti G, Sturda F, Capobianco AM, Stigliani V, et al. Preliminary clinical evaluation of free/total PSA ratio by the IMMULITE system. Int J Biol Markers 1996;11:24-8. 8) Wymenga LF, Duisterwinkel FJ, Groenier K, Visser-van Brummen P, Marrink J, Mensink HJ. Clinical implications of free-to-total immunoreactive prostate-specific antigen ratios. Scand J Urol Nephrol 2000;34:181-7. 9) McArdle PA, Potlock MA, Wallace AM, McMillan DC, Crooks JE, Underwood MA. Comparison of total, complexed and free prostate-specific antigens and their ratios in the detection of prostate cancer in a non-screened population. Ann Clin Biochem 2004;41:201-6. 10) Martinez-Pineiro L, Garcia Mediero JM, Gonzalez Gancedo P, Tabernero A, Lozano D, et al. Probability of prostate cancer as a function of the percentage of free prostate-specific antigen in patients with a non-suspicious rectal examination and total prostate-specific antigen of 4-10 ng/ml. World J Urol 2004;22:124-31. 11) Wesseling S, Stephan C, Semjonow A, Lein M, Brux B, Sinha P, et al. Determination of non-alpha1-antichymotrypsin-complexed prostate-specific antigen as an indirect measurement of free prostate-specific antigen: analytical performance and diagnostic accuracy. Clin Chem 2003;49:887-94. 12) Stephan C, Lein M, Jung K, Schnorr D, Loening SA. The influence of prostate volume on the ratio of free to total prostate specific antigen in serum of patients with prostate carcinoma and benign prostate hyperplasia. Cancer 1997 Jan;79:104-9. 13) Stamey TA, Yemoto CE. Examination of the 3 molecular forms of serum prostate specific antigen for distinguishing negative from positive biopsy: relationship to transition zone volume. J Urol 2000;163:119-26. 14) Wolff JM, Borchers H, Rohde D, Jakse G. Age related changes of free and total prostate specific antigen in serum. Anticancer Res 1999;19:2629-32. 15) Stephan C, Cammann H, Semjonow A, Diamandis EP, Wymenga LF, Lein M, et al. Multicenter evaluation of an artificial neural network to increase the prostate cancer detection rate and reduce unnecessary biopsies. Clin Chem 2002;48:1279-87. 16) Woodrum D, French C. Shamel LB. Stability of free prostate-specific antigen in serum samples under a variety of sample collection and sample storage conditions. Urology 1996; 48(6A): 33-39. 17) Woodrum D, York L. Two-year stability of free and total PSA in frozen serum samples. Urology; 1998; 52(2): 247-251. 18) Catalona WJ, Partin AW, Slawin KM, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease. JAMA 2998; 279(19):1542-1547. 19) Kuriyama M, et al. Quantitation of prostate-specific antigen in serum by a sensitive enyzme immunoassay. Cancer Res 1980;40:4658-62. 20) Stamey TA, Yang N, et al. Prostate specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 1987;317:909-16. 21) Primus FJ, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine antibody for diagnosis and therapy. Clin Chem 1988;34:261-4. 22) Hansen HJ, et al. Solving the problem of antibody interference in commercial "sandwich"-type immunoassay of carcinoembryonic antigen. Clin Chem 1989;35:146-51. 23) Schroff RJ, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. Cancer Res 1985;45:879-85. 24) CLSI. Protocols for the Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. CLSI document EP17-A Vol 24 (No 34). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA, 2004.

Technical Assistance

In the United States, contact DPC's Technical Services department.
Tel: 800.372.1782 or 973.927.2828
Fax: 973.927.4101. Outside the United States, contact your National Distributor.

The Quality System of Diagnostic Products Corporation is registered to ISO 13485:2003.

Tables and Graphs

40

Precision Table 1[a] (ng/mL)

		Within-Run		Total	
Material	Mean	SD	CV	SD	CV
Control	0.64	0.029	4.5%	0.041	6.4%
Serum	1.96	0.103	5.3%	0.123	6.3%
Serum	5.12	0.253	4.9%	0.332	6.5%
Control	8.03	0.393	4.9%	0.570	7.1%
Serum	10.51	0.495	4.7%	0.660	6.3%
Serum	22.00	0.989	4.5%	1.478	6.7%

[a] Representative of 1 site across 3 kit lots

Precision Table 2[b] (ng/mL)

		Within-Run		Total	
Material	Mean	SD	CV	SD	CV
Serum	0.84	0.036	4.3%	0.065	7.7%
Serum	1.97	0.093	4.7%	0.176	8.9%
Serum	5.22	0.242	4.6%	0.395	7.6%
Serum	10.66	0.512	4.8%	0.780	7.3%
Serum	21.89	0.943	4.3%	2.427	11.1%

[b] Representative of 3 sites (3 kit lots at 2 sites, 1 kit lot at 1site)

Specificity

Compound	Amount Added
AFP	10,000 ng/mL
CEA	100 ng/mL
Ferritin	10,000 ng/mL
HCG	100,000 mIU/mL
PAP	1,000 ng/mL
Prolactin	200 ng/mL

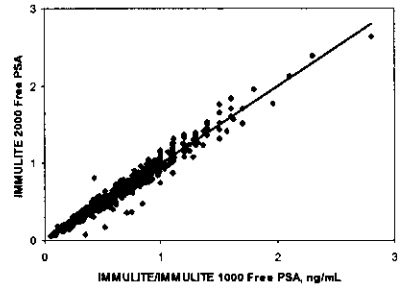
Hemolysis

	Hemoglobin					
	156 mg/dL		313 mg/dL		626 mg/dL	
	Exp	Obs	Exp	Obs	Exp	Obs
1	0.02	0.02	0.02	0.03	0.02	0.03
2	1.45	1.72	1.44	1.72	1.42	1.87
3	2.39	2.97	2.37	3.20	2.34	3.12
4	3.88	4.73	3.85	4.72	3.79	4.79
5	4.20	4.76	4.17	5.60	4.10	5.51
6	3.64	4.48	3.61	5.15	3.56	4.76
7	4.11	5.54	4.08	5.22	4.02	5.56

Linearity (ng/mL)

	Dilution	Observed	Expected	%O/E
1	16 in 16	1.17	—	—
	8 in 16	0.56	0.59	95%
	4 in 16	0.27	0.29	93%
	2 in 16	0.14	0.15	93%
	1 in 16	0.06	0.07	86%
2	16 in 16	2.42	—	—
	8 in 16	1.21	1.21	100%
	4 in 16	0.58	0.61	95%
	2 in 16	0.29	0.30	97%
	1 in 16	0.15	0.15	100%
3	16 in 16	3.71	—	—
	8 in 16	2.01	1.86	108%
	4 in 16	0.99	0.93	106%
	2 in 16	0.49	0.46	107%
	1 in 16	0.24	0.23	104%
4	16 in 16	4.34	—	—
	8 in 16	1.98	2.17	91%
	4 in 16	1.06	1.09	97%
	2 in 16	0.55	0.54	102%
	1 in 16	0.26	0.27	96%
5	16 in 16	3.26	—	—
	8 in 16	1.65	1.63	101%
	4 in 16	0.82	0.82	100%
	2 in 16	0.40	0.41	98%
	1 in 16	0.20	0.20	100%
6	16 in 16	4.65	—	—
	8 in 16	2.55	2.33	109%
	4 in 16	1.27	1.16	109%
	2 in 16	0.64	0.58	110%
	1 in 16	0.32	0.29	110%
7	16 in 16	28.95	29.00	99.8%
	8 in 16	15.20	14.50	105%
	4 in 16	7.52	7.25	104%
	2 in 16	3.63	3.63	100%
	1 in 16	1.80	1.81	99%

Method Comparison



(IML 2000) = 1.00 (IML) – 0.003 ng/mL
r = 0.98

DPC®

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2006-12-12

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42

IMMULITE® 2000

Free PSA

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English

Intended Use:

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Catalog Number: **L2KPF2** (200 tests)

Test Code: **fPS** Color: **Light Gray**

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Using assays such as these, free PSA was found to comprise a significantly ($p < 0.0001$) smaller fraction of the total PSA concentration in patients with untreated PCa than in patients with benign prostatic hypertrophy (BPH).[3]

A substantial body of literature indicates the benefit of combining free and total PSA in the form of a ratio, facilitating discrimination between PCa and BPH. These determinations must be made using assays developed by the same manufacturer.[5,6] The free-to-total ratio (% free PSA) is typically expressed as a percent: 100 × free PSA / total PSA, with both measured in ng/mL. The % free PSA is, on average, lower in PCa than in BPH and has been commonly used as an aid in PCa diagnosis when the total PSA concentration falls in the "gray zone," that is, between 4 and 10 ng/mL.[7-10] While a lower cutoff might result in fewer false-positive results, a higher cutoff is less likely to miss actual instances of PCa. The % free PSA has also been investigated in the wider total PSA concentration range of 2.0 to 20 ng/mL.[6,8,9,11]

The optimal % free PSA cutoff depends on other factors as well, one of which is the prostate and/or transition zone volume measured by transrectal ultrasound (TRUS).[12,13] Age is another factor: while both free and total PSA rise with advancing age, the % free PSA decreases.[14] More complex approaches employing artificial neural networks (ANNs) take these parameters and others into account, e.g., the results of a digital rectal exam (DRE). Using an ANN can significantly increase discrimination between BPH and PCa.[15]

Principle of the Procedure

IMMULITE 2000 Free PSA is a solid-phase, sequential chemiluminescent immunometric assay.

The solid phase (bead) is coated with monoclonal murine anti-PSA antibody specific for free (uncomplexed) PSA. The patient sample is incubated with the bead during the first cycle at which time free PSA in the sample binds to the free PSA-specific monoclonal antibody-coated bead. Unbound serum is then removed by a centrifugal wash. Alkaline phosphatase (bovine calf intestine) conjugated to a goat anti-PSA polyclonal antibody in the reagent is introduced in the second cycle and binds to free PSA on the bead to form an antibody-sandwich complex. Unbound enzyme conjugate is then removed by a centrifugal wash. Finally, chemiluminescent substrate is added to the bead and signal is generated in proportion to the bound enzyme.

Incubation Cycles: 2 × 30 minutes.

Specimen Collection

The use of an ultracentrifuge is recommended to clear lipemic samples.

Hemolyzed or grossly contaminated samples may give erroneous results.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Blood collection tubes from different manufacturers may yield differing values, depending on materials and additives, including gel or physical barriers, clot activators and/or anticoagulants. IMMULITE 2000 Free PSA has not been tested with all possible variations of tube types. Consult the section on Alternate Sample Types for details on tubes that have been tested.

Volume Required: 25 µL serum.

Storage: 24 hours at 2–8°C or for longer at –20°C.[16,17]

Warnings and Precautions

For *in vitro* diagnostic use only.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water.

Materials Supplied

Components are a matched set. The barcode labels are needed for the assay.

Free PSA Bead Pack (L2PF12)
With barcode. 200 beads, coated with monoclonal murine anti-PSA antibody specific for free (uncomplexed) PSA. Stable at 2–8°C until expiration date.
L2KPF2: 1 pack.

Free PSA Reagent Wedge (L2PFA2)
With barcodes.11.5 mL of a protein buffer matrix, with preservative; 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat anti-PSA antibody specific for PSA in a buffer containing human serum, with preservative. Stable at 2–8°C until expiration date.
L2KPF2: 1 wedge.

Before use, tear off the top of the label at the perforations, without damaging the barcode. Remove the foil seal from the top of wedge; snap the sliding cover down into the ramps on the reagent lid.

Free PSA Adjustors (LPFL, LPFH)
Two vials (Low and High) of lyophilized free PSA in a buffer solution, with preservative. Reconstitute each vial with **3.0 mL** distilled or deionized water. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved. Stable at 2–8°C for 30 days after reconstitution, or for 6 months (aliquotted) at –20°C.
L2KPF2: 1 set.

Before making an adjustment, place the appropriate Aliquot Labels (supplied with the kit) on test tubes so that the barcodes can be read by the on-board reader.

Kit Components Supplied Separately

Multi-Diluent 2 (L2M2Z, L2M2Z4)
For the on-board dilution of patient samples. One vial, concentrated (ready-to-use), nonhuman protein/buffer matrix, with preservative. Storage: 30 days (after opening) at 2–8°C or 6 months (aliquotted) at –20°C.
L2M2Z: 25 mL **L2M2Z4:** 55 mL

Barcode labels are provided for use with the diluent. Before use, place an appropriate label on a 16 × 100 mm test tube, so that the barcodes can be read by the on-board reader.
L2M2Z: 3 labels **L2M2Z4:** 5 labels

L2SUBM: Chemiluminescent Substrate
L2PWSM: Probe Wash
L2KPM: Probe Cleaning Kit
LRXT: Reaction Tubes (disposable)
L2ZT: 250 Sample Diluent Test Tubes (16 × 100 mm)
L2ZC: 250 Sample Diluent Tube Caps

TMCO: Tri-level, multi-constituent control

Also Required
Distilled or deionized water; test tubes; controls.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in the IMMULITE 2000 Operator's Manual.

See the IMMULITE 2000 Operator's Manual for: preparation, setup, dilutions, adjustment, assay and quality control procedures.

Recommended Adjustment Interval: 2 weeks.

Quality Control Samples: Use controls or sample pools with at least two levels (low and high) of free PSA.

Expected Values

A reference range study for IMMULITE 2000 Free PSA was performed at one U.S. clinical site using serum samples from 119 apparently healthy (no underlying diseases and absence of infection/fever for the previous 2–4 weeks to blood draw) male volunteers ranging in age from 50–77 years. The mean age was 61 years (SD 6.6) and the median was 60 years.

The median and 95th percentile for IMMULITE 2000 Free PSA results are tabulated below. The percentile was determined nonparametrically.

Group	Median	95%ile	Units	n
Males	0.23	0.70	ng/mL	119

Consider these limits as *guidelines* only. Each laboratory should establish its own reference ranges.

Limitations

IMMULITE 2000 free and total PSA assays should be used to calculate the % free PSA. Percent free PSA values should not be interpreted as absolute evidence for the presence or absence of malignant disease.[18]

Prediction of malignant prostatic disease should be based on a complete clinical evaluation of the patient which may also include serial serum free and total PSA determinations.

The results of the IMMULITE 2000 Free PSA assay are only valid when total PSA is in the range of 4 to 10 ng/mL.

Samples should be obtained before biopsy, prostatectomy or prostatic massage.[19]

The assay was studied in a population of 321 patients where 80% were 50-69 years of age and 62% had positive biopsies for prostate cancer. The prevalence of prostate cancer in the patients accrued for the clinical study is higher than that which occurs in the general population. Clinical performance results may not be the same in a population with lower prevalence of prostate cancer.

A sample containing approximately 1 ng/mL Free PSA was spiked with approximately 9 ng/mL complexed PSA (PSA complexed to alpha-1 antichymotrypsin). The difference in the IMMULITE family Free PSA assay result before spiking complexed PSA (1.06 ng/mL) and after spiking complexed PSA (1.14 ng/mL) was 7.5%.

The accuracy of free PSA assays cannot be evaluated by determining the recovery of free PSA spiked into serum samples, because the formation of complexes between free PSA and the PSA-binding proteins present in normal sera (α_1-antichymotrypsin and α_2-macroglobulin) will generally cause erroneously low recoveries.

Some individuals have antibodies to mouse protein which can cause interference in immunoassays that employ antibodies derived from mice. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy, in particular, may contain human anti-mouse antibodies (HAMA). These specimens may show erroneous results in such assays.[20-22] Therefore, IMMULITE 2000 Free PSA results should be used only in conjunction with results from some other diagnostic procedure and information available from the clinical evaluation of the patient.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Performance Data

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed in ng/mL. (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

Calibration Range: 0.07 to 25.0 ng/mL (WHO NIBSC 1st IS 96/668)

Analytical Sensitivity: Limit of Blank (highest value expected for a sample with no analyte; calculated as the value lying two standard deviations above that of the lowest calibrator):
0.02 ng/mL

Limit of Detection (lowest detectable concentration; determined in accordance with CLSI EP17-A[24]):
0.07 ng/mL

Functional Sensitivity: (concentration with 20% coefficient of variation (CV) determined in accordance with CLSI EP17-A[24]):
0.07 ng/mL

High-dose Hook Effect: None up to 15,118 ng/mL.

45

Precision: Samples were assayed in quaduplicate over the course of 20 days, one run per day, for a total of 20 runs and 80 replicates. (See "Precision" table.)

Linearity: The assay is linear to 25.0 ng/mL. Samples assayed under various dilutions demonstrated linearity. (See "Linearity" table for representative data.)

Specificity: IMMULITE 2000 Free PSA is highly specific for prostate-specific antigen, with a particularly low cross-reactivity to other naturally occurring compounds that might be present in patient samples including alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), ferritin, human chorionic gonadotropin (HCG), prostatic acid phosphatase (PAP), and prolactin. (See "Specificity" table.)

Bilirubin: Presence of bilirubin in concentrations up to 200 mg/L has no effect on results, within the precision of the assay.

Hemolysis: Presence of red blood cells may interfere in the IMMULITE 2000 Free PSA assay, causing small elevations in observed values. (See "Hemolysis" table.)

Lipemia: Presence of triglycerides in concentrations up to 3,000 mg/dL has no effect on results, within the precision of the assay.

Alternate Sample Type: To assess the effect of alternate sample types, blood was collected from 22 volunteers into serum glass, serum plastic and serum gel separator tubes (SST). Linear regression analysis indicates equivalence among sample/tube types as follows:

(Serum Plastic) = 0.948 (Serum Glass) + 0.0246 ng/mL
r = 0.992

(SST Plastic) = 0.938 (Serum Plastic) + 0.167 ng/mL
r = 0.991

Means:
4.61 ng/mL (Serum Glass)
4.40 ng/mL (Serum Plastic)
4.29 ng/mL (SST)

Method Comparison: IMMULITE 2000 Free PSA was compared to IMMULITE/IMMULITE 1000 Free PSA on 680 samples enrolled at 4 clinical sites (concentration range 0.05 – 2.80 ng/mL). See Method Comparison graph. Linear regression analysis indicates that free PSA assays are equivalent across IMMULITE/IMMULITE 1000 (IML) and IMMULITE 2000 (IML 2000) instruments:

(IML) = 0.97 (IML 2000) + 0.02 ng/mL
r = 0.98

Means:
0.59 ng/mL (IML 2000)
0.59 ng/mL (IML)

Clinical Performance: A study was undertaken to assess the clinical utility of the IMMULITE 2000 Free PSA assay for differentiating between benign prostatic conditions and prostate cancer, using percent free PSA determinations in 321 patients from 4 clinical sites with total PSA levels between 4–10 ng/mL and negative digital rectal examinations.

Among the 321 patients analyzed, 80% were 50–69 years of age and 62% had positive biopsies for prostate cancer.

Using the biopsy-confirmed diagnosis of prostate cancer (n = 198) versus no prostate cancer (n = 123) as the reference, Receiver Operator Characteristic Curve analysis was used to evaluate the clinical performance of the free/total PSA ratio at different diagnostic cutoffs. The area under the curve was 0.70 (95% CI = 0.64 – 0.76).

The cutoff of 20% yields a sensitivity of 95% (95% CI = 91% – 98%) and a specificity of 24% (95% CI = 16% – 32%).

The cutoff of 16% yields a sensitivity of 85% (95% CI = 79% – 90%) and a specificity of 40% (95% CI = 31% – 49%).

References

1) Chu TM, et al. Circulating antibody to prostate antigen in patients with prostatic cancer. Ann NY Acad Sci 1983;417: 383-9. 2) Zhou AM, et al. Multiple forms of prostate-specific antigen in serum: differences in immunorecognition by monoclonal and polyclonal assays. Clin Chem 1993;39:2483-91. 3) Lilja H, et al. Prostate-specific antigen in serum occurs predominantly in complex with α_1-antichymotrypsin. Clin Chem 1991;37:1618-24. 4) Christensson A, Laurell CB, Lilja H. Enzymatic activity of prostate-specific antigen and its reactions with extrecellular serine protease inhibitors. Eur J Biochem 1990;194(3):755-63. 5) Wolff JM, Stocker G, Borchers H, Haubeck H, Greiling H, Jakse G. Critical aspects related to the interpretation of the free-to-total PSA-ratio. Anticancer Res 1999;19:2633-6. 6) Patel D, White PA, Milford Ward A. A comparison of six commercial assays for total and free prostate specific antigen (PSA): the predictive value of the ratio of free to total PSA. BJU Int 2000;85:686-9. 7) Correale M, Pagliarulo A, Donatuti G, Sturda F, Capobianco AM, Stigliani V, et al. Preliminary clinical evaluation of free/total PSA ratio by the IMMULITE system. Int J Biol Markers 1996;11:24-8. 8) Wymenga LF, Duisterwinkel FJ, Groenier K, Visser-van Brummen P, Marrink J, Mensink HJ. Clinical implications of free-to-total immunoreactive prostate-specific antigen ratios. Scand J Urol Nephrol 2000;34:181-7. 9) McArdle PA, Pollock MA, Wallace AM, McMillan DC, Crooks JE, Underwood MA. Comparison of total, complexed and free prostate-specific antigens and their ratios in the detection of prostate cancer in a non-screened population. Ann Clin Biochem 2004;41:201-6. 10) Martinez-Pineiro L, Garcia Mediero JM, Gonzalez Gancedo P, Tabernero A, Lozano D, et al. Probability of prostate cancer as a function of the percentage of free prostate-specific antigen in patients with a non-suspicious rectal examination and total prostate-specific antigen of 4-10 ng/ml. World J Urol 2004;22:124-31. 11) Wesseling S, Stephan C, Semjonow A, Lein M, Brux B, Sinha P, et al. Determination of non-alpha1-antichymotrypsin-complexed prostate-specific antigen as an indirect measurement of free prostate-specific antigen: analytical performance and diagnostic accuracy. Clin Chem 2003;49:887-94. 12) Stephan C, Lein M, Jung K, Schnorr D, Loening SA. The influence of prostate volume on the ratio of free to total prostate specific antigen in serum of patients with prostate carcinoma and benign prostate hyperplasia. Cancer 1997 Jan;79:104-9. 13) Stamey TA, Yemoto CE. Examination of the 3 molecular forms of serum prostate specific antigen for distinguishing negative from positive biopsy: relationship to transition zone volume. J Urol 2000;163:119-26. 14) Wolff JM, Borchers H, Rohde D, Jakse G. Age related changes of free and total prostate specific antigen in serum. Anticancer Res 1999;19:2629-32. 15) Stephan C, Cammann H, Semjonow A, Diamandis EP, Wymenga LF, Lein M, et al. Multicenter evaluation of an artificial neural network to increase the prostate cancer detection rate and reduce unnecessary biopsies. Clin Chem 2002;48:1279-87. 16) Woodrum D, French C. Shamel LB. Stability of free prostate-specific antigen in serum samples under a variety of sample collection and sample storage conditions. Urology 1996; 48(6A): 33-39. 17) Woodrum D, York L. Two-year stability of free and total PSA in frozen serum samples. Urology; 1998; 52(2): 247-251. 18) Catalona WJ, Partin AW, Slawin KM, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease. JAMA 2998; 279(19):1542-1547. 19) Kuriyama M, et al. Quantitation of prostate-specific antigen in serum by a sensitive enyzme immunoassay. Cancer Res 1980;40:4658-62. 20) Stamey TA, Yang N, et al. Prostate specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 1987;317:909-16. 21) Primus FJ, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine antibody for diagnosis and therapy. Clin Chem 1988;34:261-4. 22) Hansen HJ, et al. Solving the problem of antibody interference in commercial "sandwich"-type immunoassay of carcinoembryonic antigen. Clin Chem 1989;35:146-51. 23) Schroff RJ, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. Cancer Res 1985;45:879-85. 24) CLSI. Protocols for the Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. CLSI document EP17-A Vol 24 (No 34). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA, 2004.

Technical Assistance

In the United States, contact DPC's Technical Services department.
Tel: 800.372.1782 or 973.927.2828
Fax: 973.927.4101. Outside the United States, contact your National Distributor.

The Quality System of Diagnostic Products Corporation is registered to ISO 13485:2003.

Tables and Graphs

Precision Table 1 [a] (ng/mL)

		Within-Run		Total	
Material	Mean	SD	CV	SD	CV
Control	0.66	0.031	4.7%	0.036	5.5%
Serum	2.02	0.077	3.8%	0.105	5.2%
Serum	5.07	0.186	3.7%	0.274	5.4%
Control	7.76	0.298	3.8%	0.459	5.9%
Serum	10.18	0.386	3.8%	0.504	5.0%
Serum	20.75	0.824	4.0%	1.162	5.6%

[a] Representative of 1 site across 3 kit lots

Precision Table 2[b] (ng/mL)

		Within-Run		Total	
Material	Mean	SD	CV	SD	CV
Serum	0.87	0.034	3.9%	0.067	7.7%
Serum	2.00	0.082	4.1%	0.125	6.3%
Serum	5.28	0.243	4.6%	0.434	8.2%
Serum	10.58	0.408	3.9%	0.811	7.7%
Serum	20.58	0.899	4.4%	1.389	6.7%

[b] Representative of 3 sites (3 kit lots at 3 sites)

Specificity

Compound	Maximum Concentration Not Interfering
AFP	10,000 ng/mL
CEA	100 ng/mL
Ferritin	10,000 ng/dL
HCG	100,000 mIU/mL
PAP	1,000 mg/L
Prolactin	200 ng/mL

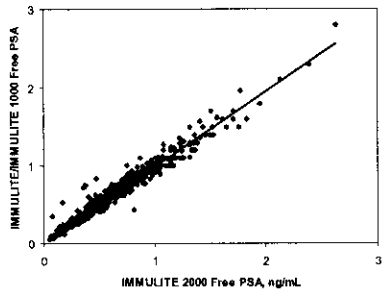
Hemolysis

	Hemoglobin					
	156 mg/dL		313 mg/dL		626 mg/dL	
	Exp	Obs	Exp	Obs	Exp	Obs
1	0.00	0.00	0.00	0.00	0.00	0.00
2	1.23	1.30	1.22	1.47	1.20	1.58
3	2.23	2.24	2.22	2.47	2.18	2.55
4	3.36	3.77	3.34	4.09	3.29	4.18
5	3.95	4.42	3.92	4.59	3.86	4.69
6	3.45	3.91	3.43	3.91	3.38	4.03
7	3.93	4.55	3.90	4.96	3.84	5.01

Linearity (ng/mL)

	Dilution	Observed	Expected	%O/E
1	16 in 16	1.08	—	—
	8 in 16	0.56	0.54	104%
	4 in 16	0.29	0.27	107%
	2 in 16	0.16	0.14	114%
	1 in 16	0.09	0.07	129%
2	16 in 16	2.30	—	—
	8 in 16	1.20	1.15	104%
	4 in 16	0.59	0.58	102%
	2 in 16	0.34	0.29	117%
	1 in 16	0.16	0.14	114%
3	16 in 16	3.71	—	—
	8 in 16	1.98	1.86	106%
	4 in 16	1.00	0.93	108%
	2 in 16	0.50	0.46	109%
	1 in 16	0.25	0.23	109%
4	16 in 16	4.25	—	—
	8 in 16	2.26	2.13	106%
	4 in 16	1.08	1.06	102%
	2 in 16	0.55	0.53	104%
	1 in 16	0.29	0.27	107%
5	16 in 16	3.41	—	—
	8 in 16	1.66	1.71	97%
	4 in 16	0.86	0.85	101%
	2 in 16	0.45	0.43	105%
	1 in 16	0.22	0.21	105%
6	16 in 16	5.35	—	—
	8 in 16	2.54	2.68	95%
	4 in 16	1.31	1.34	98%
	2 in 16	0.65	0.67	97%
	1 in 16	0.35	0.33	106%
7	16 in 16	29.92	29.00	103%
	8 in 16	15.34	14.50	106%
	4 in 16	7.41	7.25	102%
	2 in 16	3.86	3.63	106%
	1 in 16	1.98	1.81	109%

Method Comparison



(IML) = 0.97 (IIML 2000) + 0.02 ng/mL
r = 0.98

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