free PSA

Free prostate-specific antigen (free PSA- fPSA) **Elecsys® Systems 1010/2010**

1820800 **100 tests**

Caution
The Elecsys free PSA immunoassay should be used only with the Elecsys total PSA immunoassay to calculate the ratio (% fPSA) of free PSA (fPSA) to total PSA (tPSA). Use of another manufacturer's total PSA assay may result in an inappropriate population of patients selected for fPSA testing; and significantly different fPSA to tPSA ratios, cutoffs and prostate cancer probabilities than represented in the Expected Values section of this insert. Ratios must be calculated using tPSA and fPSA results both obtained on the Elecsys 1010 immunoassay Analyzer or both obtained on the Elecsys 2010 immunoassay Analyzer.
The measured fPSA value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the fPSA assay method used. Free PSA values determined on patient samples by differing testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations.
For USA: Caution: US federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and its use is restricted to, by or on the order of a physician.

Intended use
Immunoassay for the in vitro quantitative determination of free prostate-specific antigen in human serum and plasma. The Elecsys free PSA immunoassay is indicated for measurement of fPSA in conjunction with the Elecsys total PSA assay to develop a ratio (% fPSA) of fPSA to tPSA. This ratio is useful when used in conjunction with the Elecsys Total PSA test as an aid in distinguishing prostate cancer from benign prostatic conditions in men age 50 years or older who have a digital rectal examination (DRE) that is not suspicious for prostate cancer and an Elecsys total PSA value in the range 4 ng/ml to 10 ng/ml. Prostate biopsy is required for the diagnosis of prostate cancer. The electrochemiluminescence immunoassay "ECLIA" is intended for use on the Roche Elecsys 1010 and 2010 immunoassay analyzers.

Summary
Prostate-specific antigen (PSA) is a glycoprotein (molecular weight 30,000–34,000 daltons) having a close structural relationship to glandular kallikrein. It has the function of a serine protease.[1,5]
The proteolytic activity of PSA in blood is inhibited by the irreversible formation of complexes with proteinase inhibitors such as alpha-1-antichymotrypsin, alpha-2-macroglobulin and other acute phase proteins.[2,6] In addition to being present in these complexes, PSA is also present in blood in the free form, but is proteolytically inactive.[3,4]
PSA tests lack sufficient sensitivity and specificity to be considered ideal or absolutely diagnostic for screening or early detection because PSA is not specific for prostate cancer.[7] PSA is organ specific, being produced primarily by prostatic secretory epithelium, but has long been known to be elevated in non-malignant conditions such as benign prostatic hyperplasia (BPH). A number of studies have found that the %free PSA was significantly lower in patients having prostate cancer than those with benign disease or normal controls.[8,9] The ratio fPSA/tPSA has been demonstrated to improve the sensitivity and specificity in patients with tPSA values in the "gray zone" of 4–10 ng/ml.[10]
An equimolar tPSA determination is the prerequisite for reliable ratios.
In patients receiving therapy, particularly hormone withdrawal therapy, the fPSA/tPSA ratio cannot be utilized to differentiate prostate hyperplasia from cancer of the prostate.[11] Combining tests from different manufacturers to determine tPSA and fPSA can produce erroneous values, since total PSA tests may be standardized by differing methods or detect free PSA to differing degrees.

Test principle
Sandwich principle. Total duration of assay: 18 minutes.
- 1st incubation: 40 µl of sample, a biotinylated monoclonal PSA-specific antibody and a monoclonal PSA-specific antibody labeled with a ruthenium complex** react to form a sandwich complex.
- 2nd incubation: after the addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent bar code.

** Tris(2,2'-bipyridyl)ruthenium(II) complex ($Ru(bpy)_3^{2+}$)

Reagents - contents and concentrations
Elecsys free PSA reagent kit, Cat. No. 1820800 - 100 tests
M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 ml: Streptavidin-coated microparticles, 0.72 mg/ml, binding capacity: 470 ng biotin/mg microparticles; preservative.
R1 Anti-PSA Ab–biotin (gray cap), 1 bottle, 8 ml: Biotinylated monoclonal anti-PSA antibodies (mouse) 4 mg/l; phosphate buffer 100 mmol/l, pH 6.0; preservative.
R2 Anti-PSA Ab–$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 ml: Monoclonal anti-PSA antibodies (mouse) labeled with ruthenium complex 1.0 mg/l; phosphate buffer 100 mmol/l, pH 6.0; preservative.

Precautions and warnings
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste materials should be in accordance with local guidelines.

Reagent handling
The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in automatically via the reagent bar code.

Storage and stability
Store at 2–8°C.
Store the Elecsys free PSA reagent kit upright in order to ensure complete availability of the microparticles during the automatic mixing prior to use.
Stability:

unopened at 2–8°C	up to the stated expiration date
after opening	
on the Elecsys 2010	twelve weeks at 2–8°C
on the Elecsys 1010	six weeks
	four weeks (stored alternately in the refrigerator and on the analyzer - ambient temperature 20–25°C; up to 20 hours opened in total)

Specimen collection and preparation
Serum collected using standard sampling tubes, lithium heparin or tubes containing separating gel.
Plasma treated with sodium heparin, EDTA-K_3 or sodium citrate.
When sodium citrate is used, the results must be corrected by + 10%.
Stable for five days at 2–8°C, three months at -20°C. Only freeze once.[11]
For information on the stability of serum obtained with tubes containing separating gel, please note the data provided by the tube manufacturer.
Samples containing precipitates must be centrifuged before performing the assay. Do not use heat-inactivated samples.
Samples and controls stabilized with azide cannot be used.

Elecsys free PSA testing procedure
Materials provided
Cat. No. 1820800, Elecsys free PSA reagent kit for 100 tests contains:
- M Streptavidin-coated microparticles
- R1 Anti-PSA Ab–biotin
- R2 Anti-PSA Ab–$Ru(bpy)_3^{2+}$

Materials required (but not provided)
- Cat. No. 1820915, Elecsys free PSA CalSet, for 10 calibrations
- Cat. No. 1776452, Elecsys PreciControl Tumor Marker, for 2 x 3 ml each of PreciControl Tumor Marker 1 and 2
- Cat. No. 1732277 Elecsys Diluent Universal, 2 x 18 ml sample diluent
- Elecsys 1010 or 2010 analyzer
- Cat. No. 1662988, Elecsys ProCell, 6 x 380 ml system buffer
- Cat. No. 1662970, Elecsys CleanCell, 6 x 380 ml measuring cell cleaning solution
- Cat. No. 1706829, Elecsys 1010 Assay Cup, 12 x 32 reaction vessels, or Cat. No. 1706802, Elecsys 2010 Assay Cup, 60 x 60 reaction vessels
- Cat. No. 1706799, Elecsys 2010 Assay Tip, 30 x 120 pipette tips
- General laboratory equipment

Only available in the USA:
- Cat. No.1822101 Elecsys free PSA CalCheck for 3 levels.

Assay
For optimal performance of the assay it is important to follow the directions given for the analyzer used, and to check that the system's inventory of assay materials and other consumables is adequate.
Resuspension of the microparticles before use and the reading in of the test-specific parameters via the reagent bar code take place automatically. No manual input is necessary. If in exceptional cases the bar code cannot be read, enter the 15-digit sequence of numbers.
Elecsys 2010: Bring the cooled reagents to approx. 20°C and place on the reagent disk of the analyzer. Avoid the formation of foam. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.
Elecsys 1010: Bring the cooled reagents to approx. 20–25°C and place on the sample/reagent disk of the analyzer (ambient temperature 20–25°C). Avoid the formation of foam. Open bottle caps manually before use and close manually after use. Store at 2–8°C after use.

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Calibrators*
Elecsys free PSA has been calibrated against Enzymun-Test free PSA. This in turn was calibrated against the Stanford Reference Standard (100% free PSA).[11]
Every free PSA reagent set has a bar-coded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys free PSA CalSet.
Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent pack was registered on the analyzer). Renewed calibration is recommended as follows:
Elecsys 2010:
- after one month (when using the same reagent lot)
- after seven days (when using the same reagent kit on the analyzer)

Elecsys 1010:
- with every reagent kit
- after seven days (ambient temperature 20–25°C)
- after three days (ambient temperature 25–32°C)

Both analyzers:
- as required: e.g. quality control findings outside the specified range.

Calibration verification: Not necessary. The analyzer's software automatically checks the validity of the curve and draws attention to any deviations.

Quality control*
Elecsys PreciControl Tumor Marker 1 and 2 and other suitable controls.
Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per regent kit and after every calibration. The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

Calculation*
Elecsys 1010 and 2010 automatically calculate the fPSA concentration of each sample. The results are given in ng/ml.

Limitations – interference*[11]
The assay is unaffected by icterus (bilirubin < 65 mg/dl), hemolysis (Hb < 1.0 g/dl), lipemia (Intralipid < 1500 mg/dl) and biotin < 60 ng/ml (criterion: recovery within ± 10% of initial value).
In patients receiving therapy with high biotin doses (i.e. > 5 mg/day) no sample should be taken until at least 8 hours after the last biotin administration.[11]
No interference was observed from rheumatoid factor up to 1,500 U/ml.
There is no high-dose hook effect at fPSA concentrations up to 15,000 ng/ml.
In vitro tests were performed on 28 commonly used pharmaceuticals. Only flutamide at therapeutic daily dosage levels resulted in slightly depressed fPSA values.
As with all tests containing monoclonal mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse antibodies or have received them for diagnostic purposes. Elecsys free PSA contains additives which minimize these effects. In rare cases, interference due to extremely high titers of antibodies to streptavidin can occur.
For diagnostic purposes, the Elecsys free PSA findings should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring (reportable) range*[11]
0.010–50.00 ng/ml (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.010 ng/ml. Values above the measuring range are reported as > 50.00 ng/ml (or up to 1,000 ng/ml for twenty-fold diluted samples).

Dilution
Samples having fPSA concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:20 (either automatically by the Elecsys 1010/2010 or manually). The concentration of the diluted sample must be > 2.5 ng/ml. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the Elecsys 1010/2010 software automatically takes the dilution into account when calculating the sample concentration.

Expected values*[11]
A multicenter study was performed using samples from men (aged ≥ 50) referred to urologists for evaluation of prostate cancer. 1143 of the referred men had normal DRE that were not suspicious for prostate cancer (DRE normal cohort). Samples were evaluated using the Elecsys total PSA and free PSA immunoassays in parallel on the Elecsys 2010 and 1010 immunoassay Analyzers. No significant difference between the two analyzers were observed.

All patients underwent a transrectal prostate biopsy. Of the 1143 men with normal DRE, 664 men had tPSA results between 4–10 ng/ml on the Elecsys 2010 (tPSA 4–10:DRE normal cohort). The racial composition of PSA 4–10:DRE normal cohort was 84.5% Caucasian, 11.5% Black non-Hispanic, 2.6% Hispanic-Mexican, and 1.4% other. The median age was 66 years. The distribution of fPSA, tPSA, and ratio fPSA/tPSA (% fPSA) values by biopsy result for this cohort is shown in Table 1.

Table 1: PSA statistics by biopsy outcome (benign, malignant) Results Elecsys 2010 immunoassay analyzer

	Biopsy result	Number	Mean	Median	Min.	Max.	Stand. Error of Mean
fPSA ng/ml	Benign	463	1.19	1.11	0.26	4.14	0.02
	Malignant	201	1.00	0.92	0.34	2.39	0.03
	Total	664	1.13	1.06	0.26	4.14	0.02
tPSA ng/ml	Benign	463	6.10	5.68	3.95	10.00	0.07
	Malignant	201	6.42	6.10	3.95	10.00	0.11
	Total	664	6.20	5.84	3.95	10.00	0.06
% fPSA	Benign	463	19.72	19.2	5.1	53.4	0.32
	Malignant	201	16.00	15.2	5.2	35.8	0.42
	Total	664	18.60	18.0	5.1	53.4	0.27

A comparison of the mean %fPSA for the benign and malignant biopsy groups indicated that the difference is significant.

The % fPSA result may be used in evaluating the need for prostate biopsy in one of two ways:
1. The relative risk of prostate cancer in individual men may be considered, or
2. Patients may be managed using a single cutoff approach.

1. Individual Risk Assessment
There is an increased propability of detecting PCA as the PSA level increases. Of interest is that in an urologically referred cohort there is a 12% to 22% risk of PCA in men whose tPSA is < 4.0 ng/ml. The tPSA range of 4–10 ng/ml has been described in references 9 and10 as the diagnostic "gray zone". It is in this area that the % fPSA to tPSA ratio is of utility.

Table 2: Probability of detecting PCA on needle biopsy in urologically referred men with DRE results not suspicious for prostate cancer

tPSA ng/ml	Probability of PCA (%)	95% confidence interval
<4.0	17.1	12.4–21.6
4.0–10.0	30.3	26.8–33.8
>10.0	49.1	42.5–55.7

The probability of finding prostate cancer PCA with tPSA in the gray zone (4–10 ng/ml) increases with increasing age and with decreasing fPSA/tPSA ratios - see Table 3. The probabilities presented in Table 3 were estimated from a loglinear model.

Table 3: Probability of finding PCA on needle biopsy by age in years and % fPSA on Elecsys 2010

	Probability of finding PCA on needle biopsy by age in years (95% Confidence Interval)		
% fPSA ratio	50-59	60-69	≥ 70
≤ 10	49.2 (12.4-86.9)	57.5 (17.9-89.3)	64.5 (30.4-88.3)
11-18	26.9 (5.7-68.9)	33.9 (8.6-73.7)	40.8 (15.8-71.7)
19-25	18.3 (3.5-57.9)	23.9 (5.4-63.4)	29.7 (10.1-61.1)
>25	9.1 (3.1-23.7)	12.2 (4.7-28.1)	15.8 (9.0-26.1)

2. Single Cutoff
Alternatively, a single cutoff may be used for men in all age groups. Sensitivities (% of PCA detected) and specificities (% of biopsies avoided in men without PCA) for various %fPSA cutoffs are shown in Table 4. A cutoff of 25% results in the detection of 92.2% of prostate cancers and avoids unnecessary biopsy in 20.3% of men without prostate cancer. Virtually all (99%) of prostate cancers are detected with a cutoff of 30%, but only 8.7% of men without prostate cancer are spared biopsy.

Table 4: Agreement with Biopsy at Various % fPSA Cutoffs on Elecsys 2010

	Benign Biopsies			Malignant Biopsies		
%Free PSA	Number of Patients with negative biopsy identified at cutoff (total=463)	% agreement at cutoff	95% conf. interval	Number of Patients with positive biopsy identified at cutoff (total = 201)	% agreement at cutoff	95% conf. interval
23	141	30.5	(26.3-34.6)	173	86.1	(81.3-90.9)
25	94	20.3	(16.6-24.0)	186	92.5	(88.9-96.2)
27	65	14.0	(10.9-17.2)	192	95.5	(92.7-98.4)
30	41	8.9	(6.3-11.4)	199	99.0	(97.6-100.0)
53	1	0.2	(0.0-0.6)	201	100.0	(100.0-100.0)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

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Specific performance data of the test*[11]

Precision

Representative performance data on the Elecsys analyzers are given below. The results obtained in individual laboratories may differ.

Imprecision was assessed in a randomized study that included the effects of three reagent lots, three sites, and two Elecsys 1010 instruments. Two runs (four replicates each run) were performed daily for each of these configurations for ten days. Analysis of imprecision (median results for all configurations) in which the lot and site components have been combined is given in Table 5.

Table 5:

Sample	Mean ng/ml	Within-Run Imprecision Median Results SD ng/ml	Within-Run Imprecision Median Results CV %	Total Imprecision Median Results SD ng/ml	Total Imprecision Median Results CV %
PreciControl TM 1	1.81	0.035	1.9	0.110	6.1
PreciControl TM 2	13.6	0.304	2.2	0.852	6.3
Human serum pool 1	0.15	0.005	3.3	0.010	7.0
Human serum pool 2	2.28	0.069	3.0	0.168	7.4
Human serum pool 3	26.1	0.714	2.7	2.04	7.8

Analytical sensitivity (lower detection limit)

≤ 0.01 ng/ml

The detection limit represents the lowest free PSA concentration that can be distinguished from zero. It is calculated as the concentration lying two standard deviations above the lowest standard (master calibrator, standard 1 + 2 SD, intra-assay precision, n = 21).

Functional sensitivity:

Elecsys 2010: 0.02 ng/ml Elecsys 1010: 0.07 ng/ml

The functional sensitivity is the fPSA concentration that can be reproducibly measured with an interassay coefficient of variation of < 20%.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found: PAP and ACT: none; PSA-ACT 0.7%.

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* For more detailed information, please consult the operators' manuals for Elecsys 1010 and 2010, the product information on Elecsys free PSA and the package inserts for the system reagents, free PSA CalSet, PreciControl Tumor Marker and Diluent Universal.

For USA:
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