total PSA

total (free + complexed) PSA - Prostate-specific antigen (tPSA)

Elecsys® Systems 1010/2010

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1731262

100 tests

Caution
The measured tPSA 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 tPSA assay method used. tPSA 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. If there is a change in the tPSA assay procedure used while monitoring therapy, then the tPSA values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.
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
The Elecsys total PSA immunoassay, a quantitative in vitro diagnostic test for total prostate-specific antigen (tPSA) in human serum and plasma, is indicated for the measurement of total PSA in conjunction with digital rectal examination (DRE) as an aid in the detection of prostate cancer in men aged 50 years or older. Prostate biopsy is required for diagnosis of prostate cancer. The test is further indicated for serial measurement of tPSA to aid in the management of cancer patients.
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 the glandular kallikreins. It has the function of a serine proteinase.[1]
The proteolytic activity of PSA in blood is inhibited by the irreversible formation of complexes with protease inhibitors such as alpha-1-antichymotrypsin, alpha-2-macroglobulin and other acute phase proteins.[2] In addition to being present in these complexes, about 30% of the PSA present in blood is in the free form, but is proteolytically inactive.[3,4,5]
Elevated concentrations of PSA in serum are generally indicative of a pathologic condition of the prostate (prostatitis, benign hyperplasia or carcinoma).[5,7]
As PSA is also present in para-urethral and anal glands, as well as in breast tissue or with breast cancer, low levels of PSA can also be detected in sera from women. PSA may still be detectable even after radical prostatectomy.
The main areas in which PSA determinations are employed are the monitoring of progress and efficiency of therapy in patients with prostate carcinoma or receiving hormonal therapy.
The steepness of the rate of fall in PSA down to no-longer detectable levels following radiotherapy, hormonal therapy or radical surgical removal of the prostate provides information on the success of therapy.[6]
An inflammation or trauma of the prostate (e.g. in cases of urinary retention or following rectal examination, cystoscopy, coloscopy, transurethral biopsy, laser treatment or ergometry) can lead to PSA elevations of varying duration and magnitude.
The two monoclonal antibodies used in the Elecsys total PSA test recognize PSA and PSA-ACT on an equimolar basis.[7,8]

Test principle*
Sandwich principle. Total duration of assay: 18 minutes.
- 1st incubation: 20 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 total PSA reagent kit, Cat. No. 1731262 - 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, 10 ml:
Biotinylated monoclonal anti-PSA antibodies (mouse) 1.5 mg/l; phosphate buffer 100 mmol/l, pH 6.0; preservative.
R2 Anti-PSA Ab–$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 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 material 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 total 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	twelve weeks at 2–8°C
on the Elecsys 2010	eight weeks
on the Elecsys 1010	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 or tubes containing separating gel.
Plasma treated with lithium heparin, EDTA-K_2 or sodium citrate.
If sodium citrate is used, the results must be corrected by +10%.
Stable for five days at 2–8°C, six months at -20°C. Only freeze once.[12]
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 total PSA testing procedure*
Materials provided
Cat. No. 1731262, Elecsys total 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. 1731696, Elecsys total 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 analyzers
- 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. 1930346, Elecsys SysWash, 1 x 500 ml additive for wash water on Elecsys 1010 and 2010 analyzers
- Cat. No. 1298500, Elecsys SysClean, 5 x 100 ml system cleaning solution
- Cat. No. 1933159, Adapter for SysClean
- 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. 1776762 Elecsys total 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.

Calibrators*
Elecsys total PSA has been calibrated against the Stanford Reference Standard (90% PSA–ACT + 10% free PSA).[11]
Every total 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 total 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)

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Elecsys® Systems 1010/2010

English

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 reagent 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 tPSA concentration of each sample. The results are given in ng/ml.

Limitations – Interference*[12]
The assay is unaffected by icterus (bilirubin < 65 mg/dl), hemolysis (Hb < 2.2 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.[12]
No interference was observed from rheumatoid factor (up to 1,500 U/ml).
There is no high-dose hook effect at tPSA concentrations up to 17,000 ng/ml.
In vitro tests were performed on 28 commonly used pharmaceuticals. No interference with the assay was found.
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 total PSA contains additives which minimize these effects.
In rare cases, interference due to extremely high titers of antibodies to streptavidin can occur.
It is known that in rare cases PSA isoforms do exist which may be measured differently by different PSA tests. Findings of this kind have occasionally been reported for PSA tests from various manufacturers.[16,17,18]
For diagnostic purposes, the Elecsys total PSA findings should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring (reportable) range*[12]
0.002 (Elecsys 2010) or 0.006 (Elecsys 1010)–100 ng/ml (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.002/0.006 ng/ml. Values above the measuring range are reported as > 100 ng/ml (or up to 5,000 ng/ml for fifty-fold diluted samples).

Dilution
Samples having tPSA concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:50 (either automatically by the Elecsys 1010/2010 or manually). The concentration of the diluted sample must be > 2 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*[12]
Expected values in normal healthy males
The distribution of tPSA results was measured in a cohort of 395 normal healthy males aged 50–94 years.
The subsequent table presents the tPSA values as measured on the Elecsys 2010 immunoassay analyzer.

		tPSA (ng/ml)	
Age (years)	n	Median	95th Percentile
50–59	154	0.81	3.89
60–70	131	0.95	5.40
> 70	110	1.11	6.22

tPSA values in detection of prostate cancer
A multicenter cohort study was performed to demonstrate the effectiveness of the Elecsys total PSA immunoassay when used in conjunction with digital rectal examination (DRE) as an aid in the detection of prostate cancer in men 50 years of age or older. A total of 1121 serially accrued men fifty years of age or older participated in the study. The mean age of the cohort was 66.4 years (95% confidence interval = 65.9 to 66.8 years).

Distribution of tPSA values by biopsy result and digital rectal examination result
Prostate biopsy result: benign

		tPSA (ng/ml)		
DRE Result	n	Median	Minimum	Maximum
Normal	375	5.8	0.4	75.8
Abnormal	355	4.9	0.3	29.6
Total	730	5.4	0.3	75.8

Prostate biopsy result: malignant

		tPSA (ng/ml)		
DRE Result	n	Median	Minimum	Maximum
Normal	146	7.2	2.5	122.1
Abnormal	245	7.8	0.5	778.5
Total	391	7.4	0.5	778.5

Utility of tPSA in detection of prostate cancer
As shown in the table below, within this cohort of 1121 males, 391 (34.9%) prostate cancers were detected by biopsy. Abnormal digital rectal examination (DRE) results were reported for 245 (62.7%) of the 391 prostate cancers while tPSA results above 4 ng/ml were reported for 336 (85.9%) cancers for the Elecsys 2010 immunoassay analyzer. Of the 391 men diagnosed with cancer, 379 (96.9%) had either an abnormal DRE result or a tPSA value above 4.0 ng/ml.
The positive predictive value for the Elecsys total PSA immunoassay on the Elecsys 2010 immunoassay analyzer was 0.390 using 4.0 ng/ml as a cutoff.

Results for digital rectal examination and tPSA as referred to prostate cancers detected by biopsy in a cohort of:
1121 males 50 years or older referred to an urologist for prostate evaluation.

	Total	DRE+*	PSA+**	PSA+ or DRE+	PSA+ and DRE+	PSA+ and DRE-***	PSA- and DRE+****
Number of malignant prostate biopsies	391	245	336	379	202	134	43
% positive biopsies	34.9	40.8	39.0	36.5	47.5	30.7	24.6

* abnormal DRE ** tPSA value > 4 ng/ml *** normal DRE **** tPSA value < 4 ng/ml

Analysis of tPSA values was performed with Elecsys 2010 and Elecsys 1010 and gave essentially similar results.

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

Specific performance data of the test*[12]
Precision
Representative performance data on the Elecsys analyzers are given below. The results obtained in individual laboratories may differ.
Reproducibility was determined using Elecsys reagents, pooled human sera and controls in accordance with a modified protocol (EP5-T) of the NCCLS (National Committee for Clinical Laboratory Standards): 6 times daily for 10 days (n = 60). The following results were obtained:

		Intra-assay precision		Total precision	
Sample	Mean ng/ml	SD ng/ml	%CV	SD ng/ml	%CV
Human serum 1	0.30	0.005	1.8	0.007	2.4
Human serum 2	4.76	0.12	2.5	0.14	2.9
Human serum 3	51.1	1.15	2.2	1.95	3.8
PreciControl TM 1	2.33	0.06	2.5	0.06	2.7
PreciControl TM 2	17.2	0.39	2.3	0.50	2.9

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total PSA

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Analytical sensitivity (lower detection limit)
0.002 ng/ml *(Elecsys 2010)* 0.006 ng/ml *(Elecsys 1010)*
The detection limit represents the lowest tPSA 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:
0.03 ng/ml
The functional sensitivity is the tPSA 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; tPSA and PSA-ACT are recognized on an equimolar basis.

Method comparison*[12]
A comparison of Elecsys total PSA (y) with Enzymun-Test PSA (x) using clinical samples gave the following correlations:
Number of samples measured: 95

Passing/Bablok[13-15]	Linear regression
y = 0.30 + 1.03x	y = 0.60 + 1.02x
r = 0.99	r = 0.99
SD (md68) = 0.665	Sy.x = 1.32

The sample concentrations were between approx. 0.1 und 50 ng/ml.

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

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