Elecsys free PSA

cobas®

REF		Σ	SYSTEM
			cobas e 411
08828601190	08828601500	100	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 2140
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 751

Please note

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 same Elecsys platform, i.e. both obtained on either the **cobas e** 411, **cobas e** 601 or **cobas e** 602 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.

Intended use

Immunoassay for the in vitro quantitative determination of free prostate-specific antigen in human serum and plasma.

This assay 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 **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Prostate-specific antigen (PSA) is a glycoprotein (molecular weight 30000-34000 daltons) having a close structural relationship to glandular kallikrein.

It has the function of a serine protease.[1]

The proteolytic activity of PSA in blood is inhibited by the irreversible formation of complexes with proteinase inhibitors such as alpha-1-antichymotrypsin (ACT) and alpha-2-macroglobulin.[2,3] In addition to being present in these complexes, PSA is also present in blood in the free form, but is proteolytically inactive.[3]

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.[4] 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.[5,6] The ratio fPSA/tPSA has subsequently been demonstrated to improve the sensitivity and specificity in patients with tPSA values in the "gray zone" of 4-10 ng/mL.[7,8]

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. 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: 20 µL of sample, a biotinylated monoclonal PSA-specific antibody, and a monoclonal PSA-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After 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/ProCell M. 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 barcode or e-barcode.

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

Reagents - working solutions

The reagent rackpack is labeled as FPSA.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-PSA-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-PSA antibodies (mouse) 2 mg/L; phosphate buffer 100 mmol/L, pH 7.4; preservative.

R2 Anti-PSA-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Monoclonal anti-PSA antibodies (mouse) labeled with ruthenium complex 1.0 mg/L; phosphate buffer 100 mmol/L, pH 7.4; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

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Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

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 from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	6 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + coefficient of correlation ≥ 0.95.

Stable for 8 hours at 20-25 °C, 5 days at 2-8 °C, 12 weeks at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 08851964190, free PSA CalSet, 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

cobas e 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: The Elecsys free PSA assay has been standardized against the WHO Reference Standard 96/668 (100 % free PSA).

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Tumor Marker.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or µg/L).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 4912 nmol/L or < 1200 ng/mL).

Criterion: Recovery ± 0.06 ng/mL of initial value ≤ 0.5 ng/mL and within ± 10 % of initial value > 0.5 ng/mL.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

There is no high-dose hook effect at fPSA concentrations up to 15000 ng/mL.

In vitro tests were performed on 28 commonly used pharmaceuticals.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.01-50 ng/mL (defined by the Limit of Blank and the maximum of the master curve). Values below the detection limit are reported as < 0.01 ng/mL. Values above the measuring range are reported as > 50 ng/mL.

Lower limits of measurement

Limit of Blank = 0.01 ng/mL

Limit of Detection = 0.016 ng/mL

Limit of Quantitation = 0.018 ng/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Not necessary due to the broad measuring range.

Expected values

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 assay and Elecsys free PSA assay in parallel on the Elecsys 2010 immunoassay analyzer. A subset of these samples was evaluated on the MODULAR ANALYTICS E170 analyzer. No significant differences between the two platforms 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 analyzer (tPSA 4-10:DRE normal cohort). The ethnic 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)

Elecsys 2010	Biopsy result	N	Mean ng/mL	Median ng/mL	Min. ng/mL	Max. ng/mL	Stand. error of mean
fPSA	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	Benign	463	6.10	5.68	3.95	10.00	0.07
	Malignant	201	6.43	6.13	3.95	10.00	0.12
	Total	664	6.20	5.85	3.95	10.00	0.06
% fPSA	Benign	463	19.72	19.2	5.1	53.4	0.32
	Malignant	201	15.99	15.2	5.2	35.8	0.41
	Total	664	18.59	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 probability 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 6 and 7 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	12.5-21.6
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 the Elecsys 2010 analyzer

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 92.5 % 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 8.9 % of men without prostate cancer are spared biopsy.

Table 4: Agreement with biopsy at various % fPSA cutoffs on the Elecsys 2010 analyzer

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Benign biopsies			
free PSA %	Number of patients with negative biopsy identified at cutoff (total = 463)	Agreement at cutoff %	95 % confidence interval
23	141	30.5	26.3-34.9
25	94	20.3	16.7-24.3
27	65	14.0	11.0-17.5
30	41	8.9	6.4-11.8
53	1	0.2	0.0-1.2

Malignant biopsies			
free PSA %	Number of patients with positive biopsy identified at cutoff (total = 201)	Agreement at cutoff %	95 % confidence interval
23	173	86.1	80.5-90.5
25	186	92.5	88.0-95.8
27	192	95.5	91.7-97.9
30	199	99.0	96.5-99.9
53	201	100.0	98.2-100.0

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

Specific performance data

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

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.024	0.0008	3.4	0.002	6.7
Human serum 2	0.152	0.003	2.2	0.004	2.6
Human serum 3	0.850	0.016	1.8	0.020	2.3
Human serum 4	2.22	0.033	1.5	0.048	2.2
Human serum 5	9.62	0.149	1.6	0.236	2.5
Human serum 6	28.3	0.432	1.5	0.556	2.0
Human serum 7	47.8	0.767	1.6	0.938	2.0
Human serum 8	46.1	0.923	2.0	1.08	2.3
PreciControl TM[b)]1	0.974	0.016	1.6	0.023	2.4
PreciControl TM2	9.91	0.182	1.8	0.212	2.1

b) TM = Tumor Marker

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.024	0.001	4.9	0.002	6.8

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 2	0.154	0.002	1.6	0.003	2.1
Human serum 3	0.840	0.012	1.5	0.018	2.1
Human serum 4	2.19	0.027	1.2	0.046	2.1
Human serum 5	9.62	0.127	1.3	0.197	2.0
Human serum 6	27.7	0.300	1.1	0.512	1.8
Human serum 7	47.1	0.492	1.0	0.870	1.8
Human serum 8	45.7	0.798	1.7	1.00	2.2
PreciControl TM1	1.00	0.013	1.3	0.018	1.8
PreciControl TM2	10.2	0.134	1.3	0.199	1.9

Method comparison

A comparison of the Elecsys free PSA assay, REF 08828601190 (**cobas e** 601 analyzer; y) with the Elecsys free PSA assay, REF 03289788190 (**cobas e** 601 analyzer; x) gave the following correlations (ng/mL):

Number of serum samples measured: 218

Passing/Bablok[9]

$y = 0.973x + 0.005$

$\tau = 0.986$

The sample concentrations were between 0.011 and 47.3 ng/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

PAP and ACT: none; PSA-ACT 0.7 %.

References

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4 Oesterling JE. Prostate-Specific Antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J Urology 1991(5);145:907-923.

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7 Thiel RP, Oesterling JE, Wojno KJ, et al. A multicenter comparison of the diagnostic performance of free prostate-specific antigen. Urology 1996;48(6A):45-50.

8 Luderer AA, Chen YT, Soriano TF, et al. Measurement of the proportion of free to total prostate-specific antigen improves diagnostic performance of prostate-specific antigen in the diagnostic gray zone of total prostate-specific antigen. Urology. 1995 Aug;46(2):187-94.

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product

information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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