Elecsys total PSA

cobas®

REF		Σ	SYSTEM
08791732190	08791732500	300	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
TPSA	10185

Please note

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 different 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.

Intended use

This assay, a quantitative in vitro diagnostic test for total (free + complexed) 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 **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 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 (ACT) and alpha-2-macroglobulin.[2,3] Beside these complexes, about 10-30 % of the PSA present in blood occurs in the free form, but is proteolytically inactive.[3]

Autopsies have shown that prostate cancer is quite common. Among man aged 70-79 years the incidence was found to be 36-51 %. Most of these cancers are indolent i.e. without symptoms and relatively benign.[4] If PSA is measured and the result is found to be elevated, the decision on further steps must consider the possibility that the condition is indolent. Nevertheless, PSA screening has been found to reduce prostate cancer related mortality.[5] Different models have been proposed to improve the predictive accuracy of PSA measurements.[6]

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.[7,8]

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.[8]

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 assay recognize unbound PSA and PSA-ACT on an equimolar basis in the range of 10-50 % free PSA/total PSA which are the free PSA-ratios as seen in clinical practice.[9]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 12 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 II 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 **cobas** link.

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

Reagents - working solutions

The **cobas e** pack is labeled as TPSA.

M Streptavidin-coated microparticles, 1 bottle, 14.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-PSA-Ab~biotin, 1 bottle, 18.8 mL:
Biotinylated monoclonal anti-PSA antibody (mouse) 1.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

R2 Anti-PSA-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 18.8 mL:
Monoclonal anti-PSA antibody (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 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.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P273	Avoid release to the environment.
P280	Wear protective gloves.

Response:

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

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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 available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **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
on the analyzers	16 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 24 hours at 20-25 °C, 5 days at 2-8 °C, 24 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 and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators 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 08838534190, total PSA CalSet II, for 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL or
  REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- REF 07299001190, Diluent Universal, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- 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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: The Elecsys total PSA assay has been standardized against the Stanford Reference Standard/WHO 96/670 (90 % PSA -ACT + 10 % free PSA).[10,11,12]

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 **cobas e** pack 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 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Tumor Marker or PreciControl Universal.

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 **cobas e** pack, 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 effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1112 µmol/L or ≤ 65 mg/dL
Hemoglobin	≤ 1.37 mmol/L or ≤ 2200 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1500 IU/mL

Criterion: Recovery ± 0.1 ng/mL of initial value ≤ 1 ng/mL and ± 10 % of initial value > 1 ng/mL.

There is no high-dose hook effect at tPSA concentrations up to 17000 ng/mL.

Pharmaceutical substances
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special cancer drugs were tested. No interference with the assay was found.

Special cancer drugs

Drug	Concentration tested mg/L
Cyclophosphamide	1000
Cisplatin	225
5-Fluorouracil	500
Methotrexate	1000
Tamoxifen	50
Mitomycin	25
Carboplatin	1000
Etoposide	400
Flutamide	1000
Taxol	5.5
Doxorubicin	75

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.

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.[11,12,13]

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

0.006-100 ng/mL (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.006 ng/mL. Values above the measuring range are reported as > 100 ng/mL (or up to 5000 ng/mL for 50-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.006 ng/mL

Limit of Detection = 0.010 ng/mL

Limit of Quantitation = 0.014 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

Samples with tPSA concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:50 (either automatically by the analyzers 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 analyzer, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

The following data were established with the Elecsys total PSA assay on the Elecsys 2010 analyzer and can be transferred to **cobas e** 801 analyzer due to technical equivalence.

Expected values in normal healthy males

a) Studies in two clinical centers in the Netherlands and Germany with the Elecsys total PSA assay on sera from 244 healthy men of various age groups yielded the following results:

		tPSA (ng/mL)	
Age (years)	N	Median	95th percentile
< 40	45	0.57	1.4
40-49	42	0.59	2.0
50-59	107	0.75	3.1
60-69	41	1.65	4.1
≥ 70	9	1.73	4.4

b) The distribution of tPSA results was measured in a cohort of 395 normal healthy males aged 50-94 years (results of a study in the USA).

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
60-69	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 assay 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 50 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
Pathological	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
Pathological	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 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 analyzer. Of the 391 men diagnosed with cancer, 379

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(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 assay on the Elecsys 2010 analyzer was 0.390 using 4.0 ng/mL as a cutoff (malign prostate biopsy + tPSA > 4.0 ng/mL: n = 336 / tPSA > 4.0 ng/mL: n = 862).

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+[b)]	PSA+[c)]	PSA+ or DRE+	PSA+ and DRE+	PSA+ and DRE-[d)]	PSA- and DRE+[e)]
Total number	1121	600	862	1037	425	437	175
No. 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

b) abnormal DRE

c) tPSA value > 4 ng/mL

d) normal DRE

e) tPSA value < 4 ng/mL

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, samples 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 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.0211	0.00101	4.8	0.00107	5.1
Human serum 2	0.721	0.0225	3.1	0.0230	3.2
Human serum 3	3.95	0.0802	2.0	0.0892	2.3
Human serum 4	10.6	0.0616	0.6	0.156	1.5
Human serum 5	49.9	1.43	2.9	1.51	3.0
Human serum 6	86.6	0.632	0.7	1.28	1.5
Human serum 7	93.4	4.16	4.5	4.19	4.5
PreciControl TM[f)]1	3.85	0.0487	1.3	0.0583	1.5
PreciControl TM2	35.2	0.311	0.9	0.542	1.5
PreciControl U[g)]1	0.935	0.0135	1.4	0.0159	1.7
PreciControl U2	38.9	0.458	1.2	0.743	1.9

f) TM = Tumor Marker

g) U = Universal

Method comparison

A comparison of the Elecsys total PSA assay, REF 08791732190 (**cobas e** 801 analyzer; y) with the Elecsys total PSA assay, REF 07027966190 (**cobas e** 801 analyzer; x) gave the following correlations (ng/mL):

Number of serum samples measured: 190

Passing/Bablok[14]	Linear regression
y = 1.006x + 0.00867	y = 0.996x + 0.119
τ = 0.995	r = 1.00

The sample concentrations were between 0.007 and 98.3 ng/mL.

A comparison of the Elecsys total PSA assay, REF 08791732190 (**cobas e** 402 analyzer; y) with the Elecsys total PSA assay, REF 08791732190 (**cobas e** 801 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 222

Passing/Bablok[14]	Linear regression
y = 0.988x + 0.00869	y = 0.974x + 0.161
τ = 0.989	r = 1.00

The sample concentrations were between 0.008 and 95.1 ng/mL.

Analytical specificity

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

Prostatic acid phosphatase (PAP) and ACT: none; PSA and PSA-ACT are recognized on an equimolar basis.[15]

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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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