

3. **Assay Diluent A and Assay Diluent B** - Dilute 6mL of Assay Diluent A or B Concentrate(2×) with 6mL of deionized or distilled water to prepare 12 mL of Assay Diluent A or B. **The prepared working dilution can't be frozen.**
4. **Detection Reagent A and Detection Reagent B** - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with working **Assay Diluent A or B**, respectively (1:100).
5. **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600 mL of Wash Solution (1×).
6. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly.
3. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µl for once pipetting.
4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be **used only once**.
5. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals have completely dissolved.
6. Distilled water is recommended to be used to make the dilution for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

[SAMPLE PREPARATION]

1. Uscon, Inc. is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
5. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
6. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
7. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

[ASSAY PROCEDURE]

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank.

Add 100µL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 2 hours at 37°C.

2. Remove the liquid of each well, don't wash.
3. Add 100µL of **Detection Reagent A** working solution to each well. Incubate for 1 hour at 37°C after covering it with the Plate sealer.
4. Aspirate the solution and wash with 350µL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1–2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
5. Add 100µL of **Detection Reagent B** working solution to each well. Incubate for 30 minutes at 37°C after covering it with the Plate sealer.
6. Repeat the aspiration/wash process for five times as conducted in step 4.
7. Add 90µL of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate for 15 - 25 minutes at 37°C (Don't exceed 30 minutes), Protect from light. The liquid will turn blue by the addition of Substrate Solution.
8. Add 50µL of **Stop Solution** to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.

Note:

1. **Assay preparation:** Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at -20°C until the kits expiry date.
2. **Samples or reagents addition: Please use the freshly prepared Standard.** Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. **Incubation:** To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. **Washing:** The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading.
5. **Controlling of reaction time:** Observe the change of color after adding **TMB Substrate** (e.g. observation once every 10 minutes), if the color is too deep, add **Stop Solution** in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. **TMB Substrate** is easily contaminated. Please protect it from light.

[CALCULATION OF RESULTS]

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Create a standard curve on log-log graph paper, with EPCA2 concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis. Using some plot software, such as curve expert 1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

[DETECTION RANGE]

3.12-200ng/mL. The standard curve concentrations used for the ELISA's were 200ng/mL, 100ng/mL, 50ng/mL, 25ng/mL, 12.5ng/mL, 6.25ng/mL, 3.12ng/mL.

[SENSITIVITY]

The minimum detectable dose of human EPCA2 is typically less than 1.23ng/mL.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined the mean O.D. Value of 20 replicates of the zero standard added by their three standard deviations.

[SPECIFICITY]

This assay has high sensitivity and excellent specificity for detection of human EPCA2.

No significant cross-reactivity or interference between human EPCA2 and analogues was observed.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross- reactivity detection between human EPCA2 and all the analogues, therefore, cross reaction may still exist.

[ASSAY PROCEDURE SUMMARY]

1. Prepare all reagents, samples and standards;
2. Add 100 μ L standard or sample to each well. Incubate 2 hours at 37°C;
3. Add 100 μ L prepared Detection Reagent A. Incubate 1 hour at 37°C;
4. Aspirate and wash 3 times;
5. Add 100 μ L prepared Detection Reagent B. Incubate 30 minutes at 37°C;
6. Aspirate and wash 5 times;
7. Add 90 μ L Substrate Solution. Incubate 15-25 minutes at 37°C;
8. Add 50 μ L Stop Solution. Read at 450nm immediately.

[IMPORTANT NOTE]

1. Limited by the current condition and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
2. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
3. Kits from different batches may be a little different in detection range, sensitivity and color developing time. Please perform the experiment exactly according to the instruction attached in kit while electronic ones from our website (www.uscnk.us; www.uscnk.cn; www.uscnk.com) is only for information.

4. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
5. Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.
6. There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.
7. Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at $450 \pm 10\text{nm}$ wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment. For more information, please refer to the operation video (<http://www.uscnk.com/homepage/operate-elisa.htm>).
8. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
9. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.
10. Kits from different manufacturers for the same item might produce different results, since we haven't compared our products with other manufacturers.
11. Valid period: six months.
12. The instruction manual also suit for the kit of 48T, but all reagents of 48T kit is reduced by half.

[PRECAUTION]

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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total PSA

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

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REF 04641655 190

100 tests

• Indicates analyzers on which the kit can be used

Elecsys 2010	MODULAR ANALYTICS E170	cobas e 411	cobas e 601
•	•	•	•

English**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

The Elecsys total PSA immunoassay, 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 electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.

Summary

Prostate-specific antigen (PSA) is a glycoprotein (molecular weight 33000-34000 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, alpha-2-macroglobulin, and other acute phase proteins.² Beside these complexes, about 30 % of the PSA present in blood occurs 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).^{6,7}

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.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 test recognize 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: 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.

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.

a) Tri(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Reagents - working solutions

- 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 antibody (mouse) 1.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.
- R2 Anti-PSA-Ab-Ru(bpy)₃²⁺ (black cap), 1 bottle, 10 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.

Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request. Avoid the formation of foam with 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 via the respective reagent barcodes.

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 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	8 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.

Lithium heparin, K₂-EDTA, and sodium citrate plasma. When sodium citrate is used, the results must be corrected by + 10 %.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within $\pm 2 \times$ analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 5 days at 2-8 °C, 6 months at -20 °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 patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- REF 04485220190, total PSA CalSet II, for 4 x 1 mL
- REF 11776452122, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2 or

total PSA

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

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- [REF] 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
 - [REF] 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
 - [REF] 03183971122, Diluent Universal, 2 x 36 mL sample diluent
 - General laboratory equipment
 - Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer
- Accessories for Elecsys 2010 and **cobas e** 411 analyzers:
- [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, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
 - [REF] 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips
- Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 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 Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
 - [REF] 03023150001, WasteLiner, waste bags
 - [REF] 03027651001, SysClean Adapter M
- Accessories for all analyzers:
- [REF] 11298500316, 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 before use. Read in the test-specific parameters via the reagent barcode. In exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:
PreClean M solution is necessary.

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

Calibration

Traceability: This method has been standardized against the Stanford Reference Standard/WHO 96/670 (90 % PSA-ACT + 10 % free PSA).^{10,11,12} Every Elecsys total PSA reagent set has a barcoded 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 Cal/Set II.

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).

Renewed calibration is recommended as follows:

- after 1 month (28 days) 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 specified limits

Quality control

For quality control, use Elecsys PreciControl Tumor Marker 1 and 2 or Elecsys PreciControl Universal 1 and 2.

Other suitable control material can be used in addition. 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 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 limits.

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 < 1.4 mmol/L or < 2.2 g/dL), lipemia (Intralipid < 1500 mg/dL), and biotin < 246 nmol/L or < 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. No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

There is no high-dose hook effect at tPSA concentrations up to 17000 ng/mL. In vitro tests were performed on 28 commonly used pharmaceuticals. No interference with the assay was found.

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.^{13,14,15} 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

0.002 (Elecsys 2010 and **cobas e** 411 analyzers) or 0.003 (MODULAR ANALYTICS E170 and **cobas e** 601 analyzers)-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 ng/mL or < 0.003 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

Lower detection limit (LDL)

	Elecsys 2010 and cobas e 411 analyzers	MODULAR ANALYTICS E170 and cobas e 601 analyzers
LDL	0.002 ng/mL	0.003 ng/mL

The lower detection limit (LDL) is calculated as the concentration lying 2 signal standard deviations away from an analyte-free sample or from the lowest standard (repeatability, n = 21).

Limit of blank (LoB) and limit of detection (LoD)

	Elecsys 2010 and cobas e 411 analyzers	MODULAR ANALYTICS E170 and cobas e 601 analyzers
LoB	0.007 ng/mL	0.006 ng/mL
LoD	0.011 ng/mL	0.014 ng/mL

Both limit of blank and limit of detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements. The limit of blank is the 95th percentile value from n ≥ 60 measurements of one or several 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 samples having a low concentration. The limit of detection corresponds to the sample concentration which leads with a probability of 95 % to a measurement result above the limit of blank.

Dilution

Samples with tPSA concentrations above the measuring range can be diluted with Elecsys Diluent Universal. The recommended dilution is 1:50 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** 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 analyzers, the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

total PSA

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

Expected values

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:

Age (years)	N	tPSA (ng/mL)	
		Median	95 th 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.

Age (years)	N	tPSA (ng/mL)	
		Median	95 th 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

DRE result	N	tPSA (ng/mL)		
		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

DRE result	N	tPSA (ng/mL)		
		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 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 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 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).

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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

Analysis of tPSA values was performed with Elecsys 2010 analyzers. 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 accordance with a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Sample	Elecsys 2010 and cobas e 411 analyzers			Intermediate precision	
	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 TM2	17.2	0.39	2.3	0.50	2.9

§ Repeatability = within-run precision

§ TM = Tumor Marker

Sample	MODULAR ANALYTICS E170 and cobas e 601 analyzers			Intermediate precision		
	Mean ng/mL	SD ng/mL	CV %	Mean ng/mL	SD ng/mL	CV %
Human serum 1	1.12	0.02	1.4	1.12	0.04	3.2
Human serum 2	4.39	0.05	1.2	4.61	0.17	3.7
Human serum 3	27.8	0.46	1.7	27.5	0.75	2.7
PreciControl TM1	3.27	0.04	1.3	3.25	0.05	1.4
PreciControl TM2	23.3	0.32	1.4	22.9	0.36	1.6

Method comparison

A comparison of the Elecsys total PSA assay (y) with the Enzymun-Test PSA method (x) using clinical samples gave the following correlations:

Number of samples measured: 95

Passing/Bablok¹⁶

$$y = 1.03x + 0.30$$

$$r = 0.950$$

Linear regression

$$y = 1.02x + 0.60$$

$$r = 0.989$$

The sample concentrations were between approx. 0.1 and 50 ng/mL.



total PSA

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

Functional sensitivity

0.03 ng/mL

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of $\leq 20\%$.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found: PAP and ACT: none; PSA and PSA-ACT are recognized on an equimolar basis.

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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 package inserts of all necessary components.

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SPINREACT

CE

ACP

Acid phosphatase

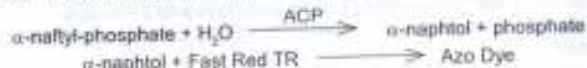
 α -Naphthyl phosphate. Kinetic

Quantitative determination of acid phosphatase (ACP) IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD

Hillmann method: Phosphatase acid activity present in the sample is determined according to the modified method of Hillmann.



α -naphthol reacts with a diazotized compound forming a colour with a maximum of absorbance at 405 nm.
Tartrate is used as specific of the prostatic fraction.

CLINICAL SIGNIFICANCE

Acid phosphatase is an enzyme present in almost all weaves of the organism, being particularly high in prostate, stomach, liver, muscle, spleen, erythrocytes and platelets.
High levels of acid phosphatase are found in prostatic pathologies as hypertrophy, prostatitis or carcinoma. In hematological disorders, bones or liver diseases as well as in Paget's or Gaucher's diseases.
Decreased serum acid phosphatase has no clinical significance.^{1,2,3}
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENTS

R 1 Buffer	Sodium citrate pH 5.2	50 mmol/L
R 2 Substrate	α -Naphthyl phosphate Fast Red TR	10 mmol/L 6 mmol/L
R 3 Tartrate	Sodium tartrate	2 mmol/L
R 4 Acetic acid		0.5 mol/L

PREPARATION

- Working reagent (WR):
Ref: 1001121.
Dissolve (→) one tablet of R 2 Substrate in one vial of R 1 Buffer.
Ref: 1001122.
Dissolve (→) one tablet of R 2 Substrate in 15 mL of R 1 Buffer.
Cap and mix gently to dissolve contents.
Stability: 2 days at 2-8°C or 8 hours at room temperature.
- R 3 and R 4: Ready to use. (R 4 Included in Ref: 1001121).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
Do not use the tablets if appears broken.
Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 450 nm \geq 0,44.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 30°C or 37°C (\pm 0,1°C)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum¹. Use only clear and unhemolyzed serum, separated from the clot as soon as possible. Do not use plasma.
Acid phosphatase is very labile; stabilize by adding 50 μ L of acetic acid (R 4) per mL of the sample. Stability: 7 days at 2-8°C.

PROCEDURE

- Assay conditions:
Wavelength: 405 nm
Cuvette: 1 cm light path
Constant temperature: 30°C / 37°C
- Adjust the instrument to zero with distilled water or air.
- Pipette into a cuvette:

	ACP Total (T)	ACP Non Prostatic (No P)
WR (mL)	1.0	1.0
R 3 (μ L)	—	50
Sample (μ L)	100	100

- Mix, incubate for 5 minute.
- Read initial absorbance (A) of the sample, start the stopwatch and read absorbance at 1 minute intervals thereafter for 3 minutes.
- Calculate the difference between absorbance and the average absorbance differences per minute ($\Delta A/\text{min}$).

CALCULATIONS

$\Delta A/\text{min} \times 750 = \text{U/L of ACP (T)}$
 $750 \times (\Delta A/\text{min ACP (T)} - \Delta A/\text{min ACP Non inhibitor by Tartrate}) = \text{U/L of ACP prostatic.}$

Units: One international unit (IU) is the amount of enzyme that transforms 1 μ mol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: SPINCONTROL H Normal and Pathologic (Ref. 1002120 and 1002210).
If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES^{4,5}

	30°C	37°C
Total acid phosphatase:		
Men:	< 4.3 U/L	< 5.4 U/L
Women:	< 3.1 U/L	< 4.2 U/L
Prostatic acid phosphatase	< 1.5 U/L	< 1.7 U/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS (Total ACP)

Measuring range: From detection limit of 0,13 U/L to linearity limit of 150 U/L.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	23,67	2,56	23,6	2,6
SD	0,22	0,07	0,22	0,07
CV (%)	0,95	2,90	0,92	2,76

Sensitivity: 1 U/L = 0.0034 A/min.

Accuracy: Results obtained using SPINREACT reagents did not show systematic differences when compared with other commercial reagents.

The results obtained using 50 samples were the following:

Correlation coefficient (r): 0,99

Regression equation: $y = 0,9977x + 0,1486$.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemolysis interferes due the high concentration of acid phosphatase in red cells¹. A list of drugs and other interfering substances with acid phosphatase determination has been reported by Young et al.^{2,3}

NOTES

SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

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PACKAGING

Ref:1001121	Cont.	15 x 2 mL
Ref:1001122		10 x 15 mL





E84215Hu 96 Tests

Enzyme-linked Immunosorbent Assay Kit

For Human Early Prostate Cancer Antigen 2 (EPCA2)

Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES

[INTENDED USE]

The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of human EPCA2 in serum, plasma and other biological fluids.

[REAGENTS AND MATERIALS PROVIDED]

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard (freeze dried)	2	Standard Diluent	1×20mL
Detection Reagent A (green)	1×120µL	Assay Diluent A (2 × concentrate)	1×6mL
Detection Reagent B (red)	1×120µL	Assay Diluent B (2 × concentrate)	1×6mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

[MATERIALS REQUIRED BUT NOT SUPPLIED]

1. Microplate reader with 450 ± 10nm filter.
2. Precision single or multi-channel pipettes and pipette tips with disposable tips.
3. Eppendorf Tubes for diluting samples.
4. Deionized or distilled water.
5. Absorbent paper for blotting the microtiter plate.
6. Container for Wash Solution

[STORAGE OF THE KITS]

All the reagents should be kept according to the labels on vials. The **Standard, Detection Reagent A, Detection Reagent B** and the **96-well strip plate** should be stored at -20°C upon being received. The unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

[TEST PRINCIPLE]

The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to EPCA2. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for EPCA2. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain EPCA2, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of $450\text{nm} \pm 10\text{nm}$. The concentration of EPCA2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

[SAMPLE COLLECTION AND STORAGE]

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately $1000\times g$. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at $1000\times g$ at $2 - 8^{\circ}\text{C}$ within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Other biological fluids - Centrifuge samples for 20 minutes at $1000\times g$. Remove particulates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at $2-8^{\circ}\text{C}$, otherwise samples must stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen can not be detected.
3. When performing the assay slowly bring samples to room temperature.

[REAGENT PREPARATION]

1. Bring all kit components and samples to room temperature ($18-25^{\circ}\text{C}$) before use.
2. **Standard** - Reconstitute the **Standard** with 1.0 mL of **Standard Diluent**, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 200ng/mL . Please prepare 7 tubes containing 0.5mL Standard Diluent and produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 200ng/mL , 100ng/mL , 50ng/mL , 25ng/mL , 12.5ng/mL , 6.25ng/mL , 3.12ng/mL , and the last EP tubes with **Standard Diluent** is the blank as 0ng/mL .

