

PROSTATIC SPECIFIC ANTIGEN (PSA)

REF 41.001.00








FINALIDADE

Kit intended to the detection and quantification of Total Prostatic Specific Antigen (PSA) in serum.

PRINCIPLE OF THE METHOD

The PSA in the serum reacts with specific monoclonal antibodies that cover the well in the microplate. The Antibody-PSA complex is recognized by monoclonal antibody anti-PSA present in the conjugate. Lately a colorimetric reaction occurs directly proportional to the PSA level in the sample, in comparison with the calibration curve.

REAGENT COMPOSITION

MPL	Polyethylene wells coated with monoclonal antibody anti human PSA.	
CONJ	Mice monoclonal antibody anti PSA conjugated with Peroxidase. Ready to use.	
TMB	Substrate solution of 3,3',5,5'-tetrametilbenzidine (TMB) and Hydrogen peroxide. Ready to use.	
STOP	Chloridric Acid solution 0.2 N. Ready to use.	
CAL	Prostatic Especific antigen (PSA) dilutions determined using WHO traceable standards 96/668 (free PSA) and 96/670 (PSA - ACT 90:10), with colorants. Concentration/Colorant: 0 ng/mL/uncolored; 0,8 ng/mL/yellow; 4 ng/mL/green; 10 ng/mL/blue. Ready to use.	

All products derived from human blood tested negative to HbsAg (Hepatitis B surface Antigen), Anti-HCV (Hepatitis C Virus) and Anti-HIV 1 and 2 (Human immunodeficiency Virus). Nevertheless, they must be treated as potentially infectious.

STORAGE AND STABILITY

- The reagents are stable up to the date stated on the label.
- Do not use reagents over the expiration date.
- Store at 2 to 8 °C.
- Do not freeze and protect from light.

MATERIAL NECESSÁRIO NÃO FORNECIDO

- Microplate ELISA reader able to read at 450 nm. It's recommended the use of a secondary filter to read at 620 to 690 nm.
- Clock or chronometer.
- Plastic assay tubes.
- 37 °C Incubator.
- Automatic microplate washer or other alternative system.
- Pipettes and Micropipettes (25µL).
- Multi-channel micropipette (75, 100 and 350 µL) and new tips.
- Absorbent paper
- Normal Saline Solution (NaCl 0.9%). To dilution of high level samples.

CUIDADOS E PRECAUÇÕES

- The kit is intended for *in vitro* diagnostic use only.
- All specimens and controls should be handled as potentially infectious.
- Wear the individual protection equipment accordingly to Good Laboratory Practice.
- Discard the reactions surplus according to Good Laboratory Practice in a proper place for potentially infective material.
- The information for Disposing, Security and First Aid are described in the Manual Safety Data Sheet (MSDS) of this product available at www.biotecnica.ind.br or calling for 55-35-3214-4646.
- Do not combine reagents from different batches.
- Do not exchange the caps from different reagents in order to avoid cross contamination.
- Do not use the reagent if it displays any sign of contamination.
- The reagents must be kept out of the specified temperatures only the time necessary for the realization of the tests.
- Use glass pipettes and disposable tips for each sample, control, standard/calibrator and reagent.
- Once initiated the assay the subsequent steps may not be interrupted.
- Avoid the formation of foam in the wells.
- Do not scratch or touch the bottom and wall of the well. It may interfere in the reaction.
- Avoid handling directly the microplate with the hands. It may stain the plate and consequently interfere the photometric reading.
- TMB solution must not be exposed to light, water, oxidants and metals.

SAMPLE - PREPARATION AND STABILITY

- Serum (Fibrin-free).
- The sample is stable for 24 hours at 2 to 8 °C and 2 months at -20 °C.
- Frozen samples should be use only once after thaw at 10 to 30 °C.

TECHNICAL PROCEDURE

A) INITIAL PROCEDURE

I) Microplate:

Let the microplate at room temperature (10 to 30 °C), before removing it from its original package.

Open the package and remove the wells that will be tested letting the unused wells in the original package with the desiccant sac. Close tightly the package and store back to 2 to 8 °C.

Execute the test with the wells fixed on the plastic rack.

II) Temperature of the reagentes to the assay:

Keep the o kit at the bench at least 30 minutes in order to achieve the room temperature. Before use, homogenize the reagents gently.

III) Prepare the **Layout** (available at www.biotecnica.ind.br) of the ELISA plate identifying the wells destined to Blank, Calibrators and each Sample to be tested.

B) PROCEDURE

Make a calibration curve at each analytical run.

It is recommended that standard and samples additions be done quickly (up to 10 minutes), in order to obtain the reproduction of the incubation time and consequently correct results.

It is recommended to execute the assay in duplicate.

I) Set apart a well to use as BLANK.

Dispense 25 µL of each standard and sample, in the appropriate wells of the microplate.

II) Immediately add 75 µL of CONJUGATE to each well.

III) Incubate de wells, sealed with the adhesive, for 1 hour at 37 °C.

IV) Wash 6 times with 250 to 350 µL of purified water in the plate washer, or manually, if a washer is not available.

Remove the liquid phase from wells. Invert the plate and hit onto dry absorbent paper.

V) Dispense 100 µL de CHROMOGEN-SUBSTRATE SOLUTION (TMB) to each well.

VI) Incubate 15 minutes, at room temperature, in the dark.

VII) Dispense 100 µL of STOP SOLUTION to each well.

VIII) Read the optical density (OD) at 450 nm, and whenever possible at a wavelength range of 620-690 nm (to minimize the interference due to plate imperfection or dirtiness).

The readings have to be done within 30 minutes after the stop reaction.

If the lab equipment does not have all recommended filters, consider only the 450 nm readings.

NOTE: Those samples which OD values are above linear range should be diluted. Normal saline solution (NaCl 0.9%) must be used as diluent. Repeat the assay and multiply the obtained result by the dilution factor.

QUALITY CONTROL OF THE ASSAY

Test will be valid provided the OD value at 450 nm for the 0 ng/ml standard is below 0.150. Higher values turn the analysis invalid. In this case, visually check the chromogen-substrate solution. A bluish color indicates that the solution is damaged due to improper handling or the expiration date has been reached. Otherwise, such result could be a consequence of improper incubation times. In this case, repeat the test strictly adhering to the working protocol outlined in this instruction sheet.

CALIBRATION/CALCULATION PROCEDURES

A = CAL/Sample absorbance at 450nm – CAL/Sample absorbance at 620-690nm
B =CAL 0 ng/mL absorbance at 450nm – CAL 0 ng/mL absorbance at 620-690nm
A – B = CAL/Sample absorbance to build the calibration curve at 450 nm.

Plot the values obtained as above for the standards versus their corresponding concentrations on graph paper (semi-log), or using an appropriate software. Plot the PSA CAL concentration in the X coordinate (logarithmic axis) and the calculated absorbance in the Y coordinate (linear axis)*.

Those samples which OD values are above linear range should be diluted. Normal saline solution (NaCl 0.9%) must be used as diluent. Repeat the assay and multiply the obtained result by the dilution factor.

* model available at www.biotecnica.ind.br

TECHNICAL LIMITATIONS

Although in the manufacture of this PSA kit maximum care has been exercised to avoid the interferences due to heterophilic antibodies, HAMA's and rheumatoid factor, potential false positive results in samples from these patients can not be ruled out.

Different values obtained with different methods and manufacturers can not be compared once they have different sensitivity and specificity.

Interfering substances

SUBSTÂNCIA	CONC.	SUBSTÂNCIA	CONC.
Folic acid	20 µg/mL	hCG	500 UI/mL
Niacine	1 mg/mL	Flutamine	500 µg/mL
Acetaminophen	1 mg/mL	Metotrexato	20 µg/mL
Aspirin	2,5 mg/mL	Ethanol	5 g/dL
Ibuprophen	2 mg/mL	Trypsin	5 µg/mL
Caffeine	500 µg/mL	Alpha-chymotrypsin	5 µg/mL
Hemoglobin	0,75 mg/mL		

CONTROLE DA QUALIDADE

Any clinical laboratory must keep an internal quality control program, which defines the aids, procedures and criteria for the tolerance limits, corrective actions and registration of the activities. Also, it must be kept a defined system for verification of the analytical variability that occurs in any measuring system.

The use of controls to evaluate the imprecision of the analysis must be a routine practice in the lab. It's suggested to use a control within the reference range and other control within the clinical significance range. The application of the Multiple Rules of Westgard for evaluation of the control state is recommended.

The lab must participate of external quality control programs.

REFERENCE RANGES

According to Urology Brazilian Society, normal range varies to age and prostate size.

Normal Values in men should be:

- Patients in the 50's: up to 2.5 ng/mL
- Patients in the 60's: up to 4.0 ng/mL

Test results should be used in association with information available from patient clinical evaluation and other diagnostic procedures.

PERFORMANCE CHARACTERISTICS

Sensitivity

The low and high quantification limits are:
0.05 ng/mL (calculated method), 0.3 ng/mL (experimental method) to 10 ng/mL.

Specificity

Monoclonal antibodies used in this test offer an unique specificity for PSA, without cross-reactions with other human serum proteins.

PSA Kit detects both equimolar form: free PSA and PSA complexed to ACT (alpha-1-antichymotrypsin), making different mixtures with standards W.H.O. free PSA 96/668 and PSA-ACT (90:10) 96/670.

All quantifications varies in a range of $\pm 15\%$.

Within Run Precision

Three repeated samples of different concentrations are tested 28 times, for the calculation from the repeatability, appreciating CV <6%.

SAMPLE	NUMBER OF REPETITION	PSA (ng/mL)	SD	CV%
1	28	1.12	0.063	5.68
2		3.56	0.144	4.25
3		8.56	0.419	4.89

Hook Effect

No hook effect has been noticed with samples up to 20,000 ng/mL. It is recommended to dilute the samples with concentration higher than linear limit.

INTERPRETATION

Prostatic-specific antigen (PSA) quantification is ordered by physicians for diagnosis and monitoring purposes in the affection of the prostate.

Benign acute prostatitis and benign prostatic hyperplasia may increase PSA levels until 10 ng/mL. Transrectal ultrasound and rectal touch may also increase PSA levels.

Results must be interpreted carefully correlated with clinical history and related therapies.

At present, specialists discuss about the right adoption of reference ranges. There are studies recommending the use of up to 4.0 ng/mL for healthy patients. Meanwhile others discuss about the use of up to 2.5 ng/mL for patients in their 60's and patients with family history of prostate cancer.

OBSERVATIONS

1. The cleaning and drying of the lab material are fundamental factors for the stability of the reagents to reach correct results.

The water used in cleaning must be recent and free of contaminating agents. The cleaning of the glassware must be done with neutral detergent. The rinsing must be exhaustive being the last ones with distilled or deionised water.

2. The water used in the lab must have the specified quality for each application. Thus, to prepare reagents the water must be of Type II, with resistivity ≥ 1 megaohms/cm or conductivity ≤ 1 microsiemens and silicate concentration < 0.1 mg/L. For the initial rinsing of the glassware, the water may be of Type III, with resistivity ≥ 0.1 megaohms/cm or conductivity ≤ 10 microsiemens. In the final rinse, the water must be of Type II. Saturated deionising columns releases alkaline water, several ions and oxidizing or reducing agents that deteriorate the reagents in a few days or even hours, changing the results in an unpredictable way. Finally, it's imperative to establish a program for the quality control of the water.

PRESENTATIONS

Presentation	Code	Components	Quantity	Symbol	Quantity
Presentation 1	Cont.	MPL CONJ TMB STOP CAL 1 to 4	1 x 96 Pcs 1 x 8 mL 1 x 12 mL 1 x 12 mL 6 x 350 μ L	Σ	96 Determ.
Presentation 2	Cont.	MPL CONJ TMB STOP CAL 1 to 4	2 X 96 Pcs 1 x 16 mL 1 x 24 mL 1 x 24 mL 6 X 700 μ L		192 Determ.

GARANTIA DE QUALIDADE

Before being approved for use BioTécnica reagents are tested in the Quality Control Department. The quality of the reagents is assured up to the expiring date stated in the label of the external packaging, since it is stored and transported in the specified conditions.

The quality control data concerning this product are available at www.biotechnicaltda.ind.br

CUSTOMER TECHNICAL SERVICE

Any technical doubt on handling this product or this procedure, contact us calling +55 35 3214 4646, your local distributor or sending an e-mail: sac@biotechnicaltda.com.br

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TECHNICAL RESPONSIBILITY

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ANVISA REGISTRATION NUMBER

80027310106

TABELA DE SÍMBOLOS INTERNACIONAIS		
SÍMBOLO	IDIOMA	DEFINIÇÃO
	Português English Español	Consultar Instruções de Uso Consult instructions for use Consultar la metódica
	Português English Español	Código Code Código
	Português English Español	Número de lote Batch code Denominación de lote
	Português English Español	Para uso diagnóstico in vitro For in vitro diagnostic medical device Para uso em diagnóstico in vitro
	Português English Español	Conteúdo do kit Contents of kit Contenido del estuche
	Português English Español	Conteúdo suficiente para <n> testes Contains sufficient for <n> tests Contenido suficiente para <n> ensayos
	Português English Español	Calibrador Calibrator Calibrador
	Português English Español	Controle Control Control
	Português English Español	Padrão Standard Patrón
	Português English Español	Reagente e seu número/abreviação Reagent and its number/abbreviation Reactivo e su número/abreviación
	Português English Español	Água purificada Purified water Agua purificada
	Português English Español	Microplaca Microplate Microplaca
	Português English Español	Conjugado Conjugate Conjugado
	Português English Español	Substrato cromógeno Chromagen substrate Substrato cromógeno
	Português English Español	Solução de parada Stop solution Solución de frenado
	Português English Español	Limite de temperatura Temperature limitation Temperatura límite
	Português English Español	Data limite de utilização (último dia do mês) Use by (last day of the month) Estable hasta (último día del mes)
	Português English Español	Fabricado por Manufactured by Elaborado por
	Português English Español	Risco biológico Biological risk Riesgo biológico
	Português English Español	Corrosivo Corrosive Corrosivo
	Português English Español	Tóxico Toxic Tóxico
	Português English Español	Inflamável Flammable Inflamable
	Português English Español	Nocivo / Irritante Harmful / Irritant Nocivo / Irritante
	Português English Español	Material reciclável Recyclable material Material reciclable
	Português English Español	Não descartar diretamente no ambiente Dispose properly Desechar adecuadamente