

Human PSA ELISA Kit

For the Quantitative Determination of Human Prostate-Specific Antigen (PSA) Concentrations in Serum.

Catalogue Number: EL10005

96 tests

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.



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INTENDED USE

This Human PSA Kit ELISA Kit is to be used for the *in vitro* quantitative determination of human Prostate-Specific Antigen (PSA) concentrations in serum. This kit is intended for LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Prostate-Specific Antigen (PSA) is a single-chain glycoprotein weighing approximately 34 kDa¹. PSA is a secretion of the prostate epithelium produced by normal, benign, and cancerous cells². PSA is functionally and immuno-chemically distinct from Prostatic Acid Phosphatase. Development of an enzyme immunoassay³ has made it possible to accurately detect low concentrations of PSA in the blood of patients with malignant and benign prostate disease. PSA serum levels are elevated in patients with prostate cancer, benign prostatic hypertrophy (BPH) and inflammatory conditions associated with the surgical stage and metastasis of the disease. After several years of clinical use, PSA has emerged as the choice overall serum marker for prostate cancer. Furthermore, numerous investigators consider PSA to be the most useful and meaningful tumour marker in cancer biology^{2,4}.

PSA is a useful serum marker for prostate cancer and represents a valuable new tool for the clinician. When used in combination with the digital rectal exam and/or transrectal ultrasound, PSA may assist in early detection programs for prostate cancer. Additionally, costly and time-consuming bone scans can be avoided for patients with newly diagnosed and untreated prostate cancer. The latter assumes a negative bone scan and therefore, a staging bone scintigram may not be necessary. Finally, an ultra-sensitive PSA assay would be useful when monitoring patients for residual disease following radical prostatectomy, as well as earlier detection of recurrent active disease following such therapies⁵.

PRINCIPLE OF THE ASSAY

This PSA enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for PSA. Standards or samples are then added to the microtiter plate wells and PSA, if present, will bind to the antibody pre-coated on the wells. In order to quantify the amount of PSA present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated monoclonal antibody, specific for PSA are added to each well to “sandwich” the PSA immobilized on the plate. The microtiter plate then undergoes incubation, followed by thorough washing of the wells to remove all unbound components. Next, a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain PSA and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450nm ± 2nm.

In order to measure the concentration of PSA in the sample, this Human PSA ELISA Kit includes a set of calibration standards (6 standards). The calibration standards are assayed at the same time as the samples allowing the operator to produce a standard curve of Optical Density (O.D.) versus PSA concentration (ng/mL). The concentration of PSA in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

| | 96 tests |
|--|-----------------|
| 1. PSA MICROTITER PLATE (Part EL05-1) _____ Pre-coated with anti-human PSA monoclonal antibody. | 96 wells |
| 2. PSA CONJUGATE (Part EL05-2) _____ Anti-human PSA polyclonal antibody conjugated to horseradish peroxidase with preservative. <i>Ready-to-use.</i> | 12 mL |
| 3. PSA STANDARD - 80 ng/mL (Part EL05-3) _____ Lyophilized human PSA in a buffered protein base with preservative that will contain 80 ng/mL after reconstitution. | 1 vial |
| 4. PSA STANDARD - 40 ng/mL (Part EL05-4) _____ Lyophilized human PSA in a buffered protein base with preservative that will contain 40 ng/mL after reconstitution. | 1 vial |
| 5. PSA STANDARD - 20 ng/mL (Part EL05-5) _____ Lyophilized human PSA in a buffered protein base with preservative that will contain 20 ng/mL after reconstitution. | 1 vial |
| 6. PSA STANDARD - 10 ng/mL (Part EL05-6) _____ Lyophilized human PSA in a buffered protein base with preservative that will contain 10 ng/mL after reconstitution. | 1 vial |
| 7. PSA STANDARD - 2 ng/mL (Part EL05-7) _____ Lyophilized human PSA in a buffered protein base with preservative that will contain 2 ng/mL after reconstitution. | 1 vial |
| 8. PSA STANDARD - 0 ng/mL (Part EL05-8) _____ Lyophilized buffered protein base with preservative that will contain 0 ng/mL after reconstitution. | 1 vial |
| 9. SUBSTRATE A (Part EL05-9) _____ Buffered solution with H ₂ O ₂ . | 10 mL |
| 10. SUBSTRATE B (Part 30007) _____ Buffered solution with TMB. | 10 mL |
| 11. STOP SOLUTION (Part 30008) _____ 2N Sulphuric Acid (H ₂ SO ₄). Caution: Caustic Material! | 15 mL |
| 12. SAMPLE DILUENT (Part EL05-10) _____ Animal serum with 0.5% proclin-300. | 10 mL |

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 μL and 50-200 μL for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Incubator ($37\pm 2^\circ\text{C}$)
8. Microtiter plate reader ($450\text{ nm}\pm 2\text{ nm}$)
9. Automatic microtiter plate washer or squirt bottle
10. Sodium hypochlorite solution, 5.25% (household liquid bleach).
11. Deionized or distilled water
12. Plastic plate cover.
13. Disposable gloves.
14. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature ($20\text{-}25^\circ\text{C}$) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at $2\text{-}8^\circ\text{C}$ in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
Solid Wastes: Autoclave for 60 minutes at 121°C .
Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat or flame.

SAMPLE PREPARATION

1. COLLECTION, HANDLING AND STORAGE

Serum: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) followed by serum extraction.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum samples to be used within 24-48 hours may be stored at 2-8°C otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay, slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

2. DILUTION PROCEDURES

If a sample is out of range, it is recommended that a 1:10 dilution be made using the Sample Diluent.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

PSA Standards: Reconstitute each PSA Standard vial with **0.6 mL** of distilled or de-ionized water. Allow each solution to sit for at least 15 minutes with gentle agitation. The PSA standard stock solutions are stable at 4°C for 3 months. Avoid freeze-thaw cycles.

Substrate Solution: Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

| Strips Used | Substrate A (mL) | Substrate B (mL) | Substrate Solution (mL) |
|---------------------|------------------|------------------|-------------------------|
| 2 strips (16 wells) | 1.5 | 1.5 | 3.0 |
| 4 strips (32 wells) | 3.0 | 3.0 | 6.0 |
| 6 strips (48 wells) | 4.0 | 4.0 | 8.0 |
| 8 strips (64 wells) | 5.0 | 5.0 | 10.0 |
| 10 strips (80wells) | 6.0 | 6.0 | 12.0 |
| 12 strips (96wells) | 7.0 | 7.0 | 14.0 |

ASSAY PROCEDURE

1. Prepare all PSA Standards before starting assay procedure (see Preparation Reagents). *It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.*
2. Secure the desired number of coated wells in the holder, then add 50 μL of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate. Add 50 μL of Sample Diluent to each well. **IMPORTANT: COMPLETE MIXING SHOULD BE ACHIEVED BEFORE PROCEEDING.** Cover and incubate for **30 minutes at 37°C.**
3. Wash the Microtiter Plate using one of the specified methods indicated below:

Manual Washing: Remove the incubation mixture by aspirating the contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with de-ionized or distilled water, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes.** After final wash, invert plate and blot dry by hitting the plate onto absorbent papers or paper towels until no moisture appears. *Note:* Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in the frame.

Automated Washing: Aspirate all wells and wash plates **FIVE times** using distilled or de-ionized water. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL /well/wash (range: 350-400 μL). After final wash, invert plate and blot dry by hitting the plate onto absorbent papers or paper towels until no moisture appears. *It is recommended that the washer be set for soaking time of 10 seconds or shaking time of 5 seconds between washes.*
4. Add 100 μL of Conjugate into each well. Cover and incubate for **30 minutes at 37°C**
5. Repeat wash procedure as described in Step 3.
6. Add 100 μL Substrate Solution to each well. Cover and incubate for **15 minutes at 37°C.**
7. Add 100 μL of Stop Solution to each well. Mix well.
8. Read the Optical Density (O.D.) at 450nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

This standard curve is used to determine the amount of PSA in an unknown sample. The standard curve is generated by plotting the average O.D. (450nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding PSA concentration (ng/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the zero-standard (0 ng/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of PSA in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding PSA concentration. If samples generate values greater than the highest standard, dilute the samples with the Sample Diluent and repeat the assay. The concentration read from the standard curve must be multiplied by the dilution factor.

INTERPRETATION OF THE RESULTS

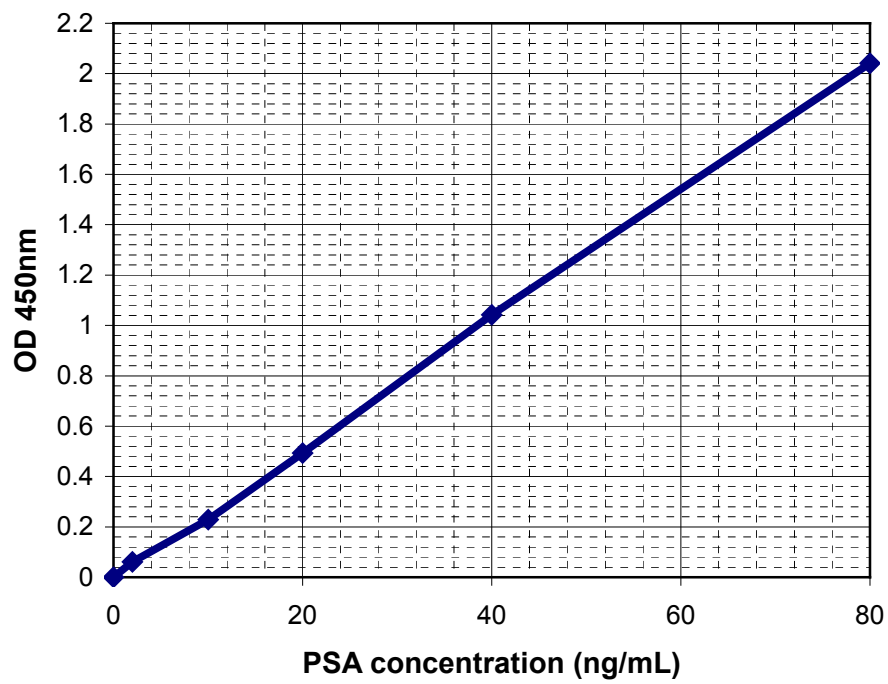
1. Samples with a PSA concentration less than 4 ng/mL are considered to be NEGATIVE by the criteria of this PSA ELISA kit. However, if there is a clinical suspicion of the presence of PSA antigens, a re-testing on a new sample is advised.
2. Samples with a PSA concentration greater than 4 ng/mL are considered to be POSITIVE by the criteria of this PSA ELISA kit. These samples should be re-tested in duplicate, using the original sample, before final confirmation of the result.
3. Initially reactive samples which are not positive in either of the duplicate repeat tests are considered to be NEGATIVE for PSA. Further testing is not required.
4. Samples repeatedly found to be POSITIVE are interpreted to contain Prostate-Specific Antigens (PSA) by the criteria of this PSA ELISA Kit.
5. Some samples may be out of range on the first assay run. Using Sample Diluent, dilute these samples to 1:10.

TYPICAL DATA

Results of a typical standard run of PSA ELISA are shown. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variations in the results. The following examples are for the purpose of illustration only, and should not be used to calculate the user's results.

EXAMPLE

| Standard (ng/mL) | O.D. (450 nm) | Mean | Zero Standard Subtracted |
|------------------|---------------|-------|--------------------------|
| 0 | 0.050, 0.048 | 0.049 | 0 |
| 2 | 0.112, 0.108 | 0.110 | 0.061 |
| 10 | 0.276, 0.280 | 0.278 | 0.229 |
| 20 | 0.540, 0.544 | 0.542 | 0.493 |
| 40 | 1.090, 1.092 | 1.091 | 1.042 |
| 80 | 2.092, 2.088 | 2.090 | 2.041 |



PERFORMANCE CHARACTERISTICS

1. PRECISION

Within-run coefficients of variation (cv) are 2.5 - 4.05%.
Between-run coefficients of variation (cv) are 4.0 - 4.5%.

2. SENSITIVITY

Estimated to be 1 ng/mL PSA antigen in human serum.

3. SPECIFICITY

No cross-reaction can be found in our assay.

4. RECOVERY

The recovery range was 95 - 100%.

5. HOOK EFFECT

In this assay, no hook effect occurs until the concentration of PSA reaches 10,000 ng/mL.

6. EXPECTED NORMAL VALUES

Each laboratory must establish its own normal range of values based on patient populations. Differences in assay techniques and the use of various standards may affect the results. The expected PSA normal value of this kit is less than 4.0 ng/mL.

7. CALIBRATION

This immunoassay is calibrated against NIBSC/WHO PSA First International Standard (Code 96/670).

LIMITATIONS OF THE PROCEDURE

This PSA ELISA PROCEDURE and the INTERPRETATION OF RESULTS sections must be closely followed when testing for the presence of PSA Antigens in serum from individual subjects. Because the PSA ELISA was designed to test individual units of serum, most data regarding its interpretation were derived from testing individual samples. Sufficient data is not available to interpret tests performed on other body specimens, pooled blood or processed plasma, and products made from such pools. Testing of these specimens is not recommended.

Serum PSA measurement is not an absolute test for malignancy. The PSA value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures. A person whose serum or plasma is found to be reactive using both ELISA and additional, more specific tests for PSA Antigens is presumed to have PSA antigens present in their serum. Appropriate counselling and medical examination should be offered. Such an evaluation should be considered an important part of PSA testing and should include test result confirmation on a freshly drawn sample.

Failure to add such a sample in the ASSAY PROCEDURE could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion for the presence of PSA Antigens.

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