Human PSA (Kallikrein-3) ELISA Kit

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

Catalogue Number: EL10005

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (02/15-05) PSA

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INTENDED USE

This Human PSA Kit ELISA Kit is to be used for the *in vitro* quantitative determination of human PSA concentrations in serum and cell culture samples. This kit is intended for **LABORATORY RESEARCH USE ONLY** and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Commonly known as Prostate-Specific Antigen (PSA), Kallikrein-3 is a peptidase that is produced in large amount by epithelial cells of the male prostate gland and female paraurethral duct during ejaculation. The protein can be produced by normal, benign, and cancerous cells. In circulation, majority of kallikrein-3 forms complex with a1-anti-chymotrypsin, with minor amount binding to other proteins. Kallikrein-3 serum levels are elevated in patients with prostate cancer, benign prostatic hypertrophy (BPH) and inflammatory conditions associated with the tissue. Kallikrein-3 has been chosen as serum marker for prostate cancer screening for males over 50 years older. Recently, this application has been challenged because its potential benefit may be out-weighted by over-diagnosis and overtreatment. In molecular biological research, it was found that the expression of Kallikrein-3 is upregulated by androgen receptor signalling pathway and down-regulated by EGF signalling pathway. The integrity of androgen receptor pathway can be monitored by measuring kallikrein-3 concentrations in biological concentrations and cell supernatant.

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) ***96 wells***
   Pre-coated with anti-human PSA monoclonal antibody.
2. **PSA CONJUGATE** (Part EL05-2) ***12 mL***
   Anti-human PSA polyclonal antibody conjugated to horseradish peroxidase with preservative. *Ready-to-use.*
3. **PSA STANDARD - 80 ng/mL** (Part EL05-3) ***1 vial***
   Lyophilized human PSA in a buffered protein base with preservative that will contain 80 ng/mL after reconstitution.
4. **PSA STANDARD - 40 ng/mL** (Part EL05-4) ***1 vial***
   Lyophilized human PSA in a buffered protein base with preservative that will contain 40 ng/mL after reconstitution.
5. **PSA STANDARD - 20 ng/mL** (Part EL05-5) ***1 vial***
   Lyophilized human PSA in a buffered protein base with preservative that will contain 20 ng/mL after reconstitution.
6. **PSA STANDARD - 10 ng/mL** (Part EL05-6) ***1 vial***
   Lyophilized human PSA in a buffered protein base with preservative that will contain 10 ng/mL after reconstitution.
7. **PSA STANDARD - 2 ng/mL** (Part EL05-7) ***1 vial***
   Lyophilized human PSA in a buffered protein base with preservative that will contain 2 ng/mL after reconstitution.
8. **PSA STANDARD - 0 ng/mL** (Part EL05-8) ***1 vial***
   Lyophilized buffered protein base with preservative that will contain 0 ng/mL after reconstitution.
9. **SUBSTRATE A** (Part EL05-9) ***10 mL***
   Buffered solution with H_20_2.
10. **SUBSTRATE B** (Part 30007) ***10 mL***
    Buffered solution with TMB.
11. **STOP SOLUTION** (Part 30008) ***15 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!
12. **SAMPLE DILUENT FOR SERUM SAMPLES** (Part EL05-10) ***10 mL***
    Animal serum with 0.5% proclin-300.

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±2°C)
8. Microtiter plate reader (450 nm±2 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 (20-25°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 2-8°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

SAMPLE DILUTION

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 (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	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 PROCEDING. 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.

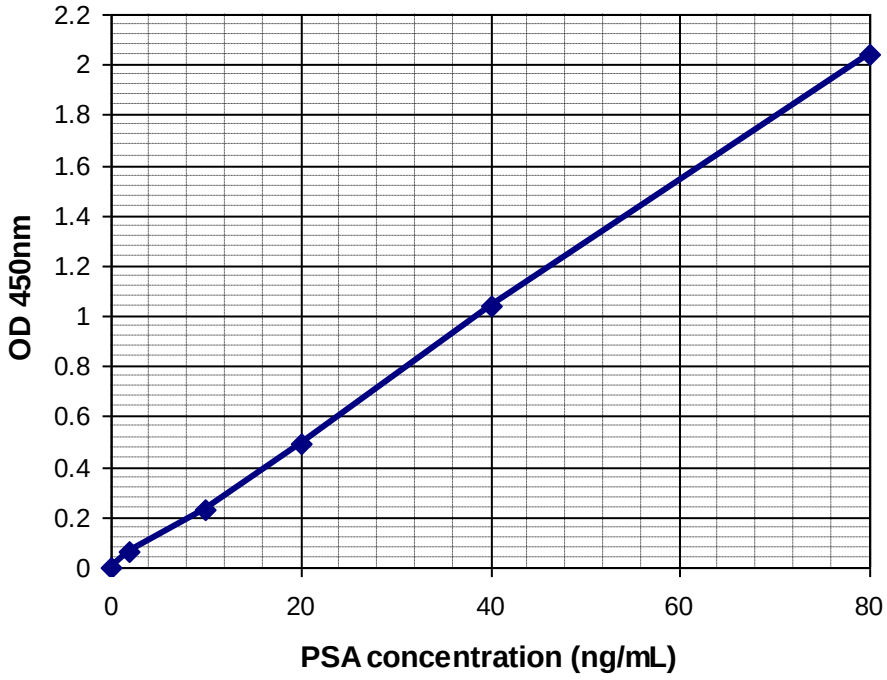
1. First, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the zero-standard (0 ng/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of PSA in each sample, 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.

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

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