Your #1 source for antibodies!

Human Free PSA ELISA Kit

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

Catalog # MBS590044

96 tests

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

S7.5(02) f-PSA

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INTENDED USE

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

INTRODUCTION

Prostate Specific Antigen (PSA) is a 33 KD single-chain serine proteinase, mainly secreted by the prostate gland (7). PSA serum levels are abnormally elevated in patients with prostate cancer, benign prostatic hypertrophy (BPH) and patients with prostate inflammatory conditions. PSA exists in serum in at least 3 different forms: free PSA, α-2 macroglobulin bounded PSA and α-1-anti-chymotrypsin bounded PSA (5). In combination with the digital rectal exam and/or transrectal ultrasound, PSA level has been used as a standard marker for early prostate cancer screening program.

Free PSA is the unbound form of prostate specific antigen (PSA). Studies (5, 6) have suggested that the percentage of free PSA in total PSA is lower in patients with prostate cancer than those with benign prostate hyperplasia. The free to total PSA ratio is now being introduced and studied as an additional tool to help clinician to decide if a patient needs more aggressive evaluation, such as prostate biopsy, to check for prostate cancer.

PRINCIPLE OF THE ASSAY

This free PSA enzyme linked immunosorbent assay (ELISA) kit uses a technique called quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for free PSA. Standards or samples are then added to the microtiter plate wells and free PSA, if present, will bind to the antibody pre-coated on the wells.

In order to quantify the amount of free PSA present in the sample, a preparation of horseradish peroxidase (HRP)-conjugated PSA antibody is added to each well. The conjugated antibody will bind to the free PSA immobilized on the plate after incubation. All unbound components will be removed by subsequent washing procedure. Next, 3, 3', 5, 5' tetramethyl-benzidine(TMB), a substrate for HRP is added to each well. The colorimetric reaction is proportional to the free PSA content in each well. The reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at the wavelength of 450nm ± 2nm.

In order to measure the concentration of free PSA in samples, this Human Free PSA ELISA Kit includes a set of calibration standards (7 standards). The calibration standards should be assayed at the same time as the samples to allow the operator to produce a standard curve of Optical Density (O.D.) versus free PSA concentration (ng/mL). The concentration of free 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. **FREE PSA MICROTITER PLATE** (Part EL50-1) ***96 wells***
   Microtiter plate pre-coated with anti-human free PSA monoclonal antibody.
2. **FREE PSA CONJUGATE** (Part EL50-2) ***12 mL***
   Anti-human free PSA antibody conjugated to horseradish peroxidase with preservative. *Ready-to-use.*
3. **FREE PSA STANDARD - 10 ng/mL** (Part EL50-3) ***1 vial***
   Lyophilized human free PSA in a buffered protein matrix contains 10 ng/mL of free PSA after reconstitution.
4. **FREE PSA STANDARD - 5 ng/mL** (Part EL50-4) ***1 vial***
   Lyophilized human free PSA in a buffered protein matrix contains 5 ng/mL of free PSA after reconstitution.
5. **FREE PSA STANDARD - 2 ng/mL** (Part EL50-5) ***1 vial***
   Lyophilized human free PSA in a buffered protein matrix contains 2 ng/mL of free PSA after reconstitution.
6. **FREE PSA STANDARD - 1 ng/mL** (Part EL50-6) ***1 vial***
   Lyophilized human free PSA in a buffered protein matrix contains 1 ng/mL of free PSA after reconstitution.
7. **FREE PSA STANDARD - 0.5 ng/mL** (Part EL50-7) ***1 vial***
   Lyophilized human free PSA in a buffered protein matrix contains 0.5 ng/mL of free PSA after reconstitution.
8. **FREE PSA STANDARD - 0.1 ng/mL** (Part EL50-8) ***1 vial***
   Lyophilized human free PSA in a buffered protein matrix contains 0.1 ng/mL of free PSA after reconstitution.
9. **FREE PSA STANDARD - 0 ng/mL** (Part EL50-9) ***1 vial***
   Lyophilized buffered protein matrix contains 0 ng/mL of free PSA after reconstitution.
10. **SUBSTRATE A** (Part EL50-10) ***10 mL***
    Buffered solution with H_2O_2
11. **SUBSTRATE B** (Part 30007) ***10 mL***
    Buffered solution with TMB.
12. **STOP SOLUTION** (Part 30008) ***10 mL***
    2N Sulphuric Acid (H_2SO_4). Caution : Caustic Material!
13. **SAMPLE DILUENT** (Part EL50-11) ***10 mL***
    Animal serum with preservative.

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.
15. 37°C incubator.

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

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.

FREE PSA Standards: Reconstitute each free PSA Standard vial with **0.5 ml** of distilled or de-ionized water. Allow each solution to sit for at least 15 minutes with gentle agitation. The reconstituted free PSA standards are stable at 4°C for at least 1 month. 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 free PSA Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.*

2. Secure a desired number of strips from the coated microtiter plate to the holder and add 50 µL of Sample Diluent to each well. Add 50 µl Standards or Samples to the appropriate wells. IMPORTANT: COMPLETE MIXING SHOULD BE ACHIEVED BEFORE PROCEDING. Cover and incubate for **45 minutes at 37°C**.

3. Wash the Microtiter Plate using one of the specified methods described 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 deionized 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 paper or paper towel until no moisture is visible. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain 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 paper or paper towel until no moisture is visible. *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 **45 minutes at 37°C**

5. Repeat wash procedure as described in Step 3.

6. Add 100 µL freshly mixed 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 10 minutes.

CALCULATION OF RESULTS

This standard curve is used to determine the amount of free 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 free 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 (0ng/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of free 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 free 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

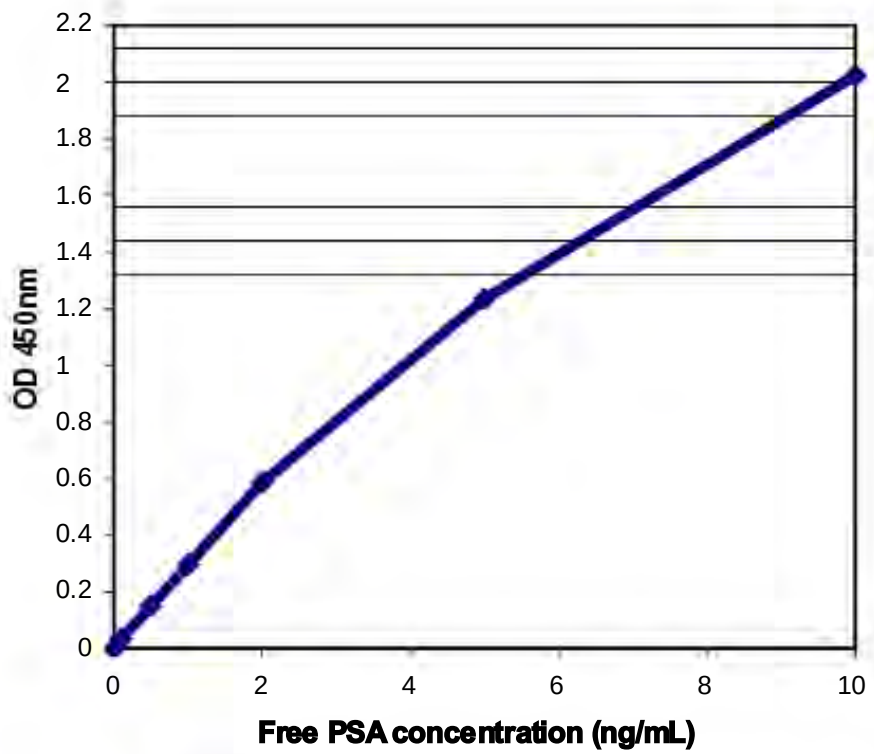
Studies have shown that the percentage of free PSA in total PSA is lower in men with prostate cancer comparing to those without cancer. The cut-off level of the percentage of free to total PSA, varies from 10% to 24% in different studies (5, 6). Setting a cut-off value of free PSA to total PSA level for prostate cancer detection has been a controversial issue (6, 8). It is very important for clinician to consider multiple factors, before reach a conclusion.

TYPICAL DATA

Results of a typical standard run of free 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. To calculate the results of samples, a standard curve must run using the same lot of reagents

EXAMPLE

Standard (ng/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted
0	0.064, 0.065	0.065	0.000
0.1	0.093, 0.105	0.099	0.035
0.5	0.212, 0.218	0.215	0.150
1	0.361, 0.361	0.361	0.296
2	0.651, 0.651	0.653	0.587
5	1.300, 1.302	1.301	1.236
10	2.091, 2.088	2.089	2.024



PERFORMANCE CHARACTERISTICS

1. **PRECISION**

Within-run coefficients of variation (CV) were determined by 8 replicate tests of three serum samples in one assay.

Samples	Mean Concentration (ng/mL)	Coefficients of Variation (CV)
1	0.086	4.79%
2	1.242	2.56%
3	10.240	1.32%

Between-run coefficients of variation (CV) were determined by testing 3 serum samples in 6 different assays.

Samples	Mean Concentration (ng/mL)	Coefficients of Variation (CV)
1	0.070	16%
2	1.233	1.8%
3	9.898	2.5%

2. **SENSITIVITY**

The sensitivity of this free PSA ELISA is estimated to be 0.022ng/mL free PSA antigen in human serum based on 3SD from the average of 16 zero standards.

3. **SPECIFICITY**
   Reacted with human free PSA, but did not react with PSA complex. The information of other cross reactions are not available.

4. **RECOVERY**
   The recovery range was 95 - 115%.

5. **HOOK EFFECT**
   In this assay, no hook effect was observed in this assay.

6. **NORMAL VALUES**
   Free PSA normally exists in minute amount in bloodstream. Over 50 serum and plasma samples from normal blood donors were tested using this assay. The free PSA contents were less than 0.5ng/mL. Free PSA level increases in aged male population and prostate inflammatory and hyperplasia individuals. The percentage of free PSA in total PSA is used in research and clinics as a way to distinguish malignant hyperplasia from other prostate abnormal conditions.

7. **CALIBRATION**
   This immunoassay is calibrated against NIBSC/WHO free PSA First International Standard (Code 96/668).

LIMITATIONS OF THE PROCEDURE

This free PSA ELISA PROCEDURE and the INTERPRETATION OF RESULTS sections must be closely followed when testing for the presence of free PSA Antigens in serum from individual subjects.

The free PSA value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures for malignancy. 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.

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