Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

PSA (Prostate Specific Antigen) ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIAPSA1	PSA Elisa	96 Tests

Intended Use:

PSA ELISA is intended to be used for the quantitative determination of PSA in human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

Human prostate-specific antigen (PSA) is a serine protease, a single chain glycoprotein with a molecular weight of approximately 34,000 daltons containing 7% carbohydrate by weight. PSA is immunologically specific for prostatic tissue, it is present in normal, benign hyperplastic, and malignant prostatic tissue, in metastatic prostatic carcinoma, and also in prostatic fluid and seminal plasma. PSA is not present in any other normal tissue obtained from men, nor is it produced by cancers of the breast, lung, colon, rectum, stomach, pancreas or thyroid. Besides, it is functionally and immunologically different from prostatic acid phosphatase (PAP).

Elevated serum PSA concentrations have been reported in patients with prostate cancer, benign prostatic hypertrophy, or inflammatory conditions of other adjacent genitourinary tissues, but not in apparently healthy men, men with non-prostatic carcinoma, apparently healthy women, or women with cancer. Reports have suggested that serum PSA is one of the most useful tumor markers in oncology. It may serve as an accurate marker for assessing response to treatment in patients with prostatic cancer. Therefore, measurement of serum PSA concentrations can be an important tool in monitoring patients with prostatic cancer and in determining the potential and actual effectiveness of surgery or other therapies. Recent studies also indicate that PSA measurements can enhance early prostate cancer detection when combined with digital rectal examination (DRE).

The PSA EIA test is a solid phase two-site immunoassay. Rabbit anti-PSA is coated on the surface of the microtiter wells and another anti-PSA monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The PSA molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme tracers are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by a colorimetric reaction. The intensity of the color formed is proportional to the concentration of PSA present in the sample.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with monoclonal anti-PSA antibody. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable until expiry date at 2-8°C.
PSA Calibrators	6x1ml	6 vials containing PSA at concentrations of 0.0, 2.5, 5.0, 15, 30 and 60 ng/ml made up in a human serum matrix. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable until expiry date at 2-8°C.
Enzyme Conjugate	1x12ml	1 vial containing 12ml of HRP labelled monoclonal Anti-PSA antibody in Buffered saline. Once open, stable until expiry date at 2-8°C.
Wash Buffer Concentrate (50X)	1x15ml	PBS-Tween at pH 7.4. 50X concentrate. The concentrate must be diluted with 735ml of distilled water before use. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x12ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable until expiry date at 2-8°C.
Stop Solution	1x12ml	Ready to use. Once open, stable until expiry date at 2-8°C.

Plastic Sealable bag, IFU and plate covers.

Materials required but not provided:

Distilled water, Vortex mixer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection:

Serum should be prepared from whole blood specimen obtained by acceptable medical techniques. The kit is for use with serum samples without additives only.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag provided and return to 2-8°C, under which conditions the wells will remain stable until the labelled expiry date. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained until the labelled expiry date.

Procedure:

Reagent preparation:

1. Bring all reagents to room temperature (18-22°C) prior to use.
2. Dilute the wash buffer concentrate with 735ml of Distilled water (yielding a total volume of 750ml). Once diluted the wash solution is stable for 2 months at room temperature. Mix well before use.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series.

STEP 2

Addition of Samples and calibrators: Add 25 µl of Calibrators and Samples to each well.

STEP 3

Addition of Enzyme Conjugate: Add 100 µl of the Enzyme Conjugate to each well. Shake the plate for 5 seconds to ensure that the added components are well mixed.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 45 minutes at room temperature (18-22°C).

STEP 5

Washing: At the end of the incubation period, remove the plate cover and discard the contents of the wells. Wash each well 5 times with diluted washing buffer of 350 µl. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of the Substrate: Add 100 µl of Substrate Solution to each well. Mix gently for 5 seconds.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 15 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 8

Stopping the Reaction: Add 100 µl of Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to yellow from blue.

STEP 9

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader within 15 minutes of adding the Stop Solution. Note down the absorbances.

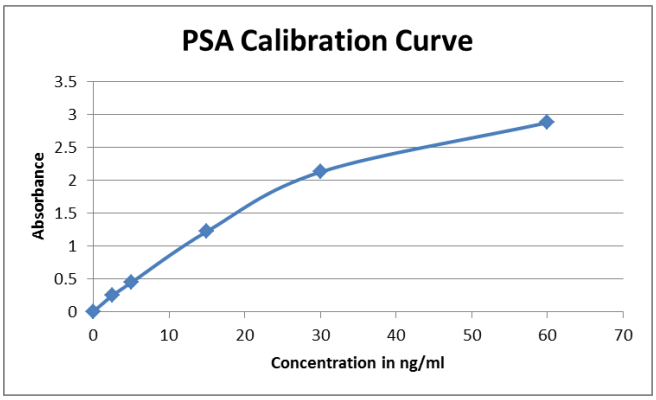
Note: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. It is recommended that no more than 32 wells are used for each assay run if manual pipetting is used since pipetting of all calibrators, specimens, controls should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available. Duplication of calibrators and specimens although not required is recommended.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in ng/ml in X axis.
- Draw a point to point curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	0.005	0.0 ng/ml
CAL B	0.246	2.5 ng/ml
CAL C	0.444	5.0 ng/ml
CAL D	1.223	15.0 ng/ml
CAL E	2.127	30.0 ng/ml
CAL F	2.879	60.0 ng/ml



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Expected Values:

Healthy males are expected to have PSA values below 4.0 ng/ml.

Sensitivity:

The minimum detectable concentration of PSA by this assay was found to be 0.5 ng/ml.

References:

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REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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