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Product Information

Total PSA (Prostate Specific Antigen) ELISA Kit

Catalog Number: EA101093
Storage Temperature: 2 – 8°C

Instruction for Use

THIS KIT IS INTENDED FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

1 INTENDED USE

The total PSA ELISA is used for measurement of total prostate specific antigen (t-PSA) in human serum or plasma (EDTA, lithium heparin or citrate) samples.

2 PRINCIPLE OF THE ASSAY

This assay is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody from mouse, directed towards an epitope of an antigen molecule (PSA). During incubation, the PSA molecule in the added samples bind to the immobilized antibody. The added enzyme conjugate, which contains an anti-PSA antibody conjugated to horseradish peroxidase, binds to the PSA forming a sandwich complex.
After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of color developed is proportional to the antigen concentration in the sample.
The measured ODs of the standards are used to construct a calibration curve against which the unknown samples are calculated.

3 MATERIALS PROVIDED WITH THE KIT

Each kit contains reagents sufficient for 96 determinations.

1. ***Microtiterwells***, 12 x 8 (break apart) strips, 96 wells.
   Wells coated with anti-PSA antibody (monoclonal).
2. ***Zero Standard,*** 1 vial, 10.0 mL, ready to use.
   (Sample Diluent)
   Contains non-mercury preservative.
3. ***Standard (Standard 1-5),*** 5 vials, 0.5 mL, ready to use;
   Concentrations: 1.56 – 3.12 – 6.25 – 12.5 – 25.0 ng/mL
   The standards are calibrated against the WHO Standard 96/670;
   Contain non-mercury preservative.
4. ***Control Low & High***, 2 vials, 0.5 mL each, ready to use;
   For control values and ranges please refer to vial label or QC-Datasheet.
   Contain non-mercury preservative.

5. ***Enzyme Conjugate***, 1 vial, 12 mL, ready to use,
   Anti-PSA antibody conjugated to horseradish peroxidase;
   Contains non-mercury preservative.
6. ***Substrate Solution***, 1 vial, 12 mL, ready to use,
   Tetramethylbenzidine (TMB).
7. ***Stop Solution***, 1 vial, 14 mL, ready to use,
   contains 0.5 M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.

4 MATERIAL REQUIRED BUT NOT PROVIDED

- Precision micropipettes (volume: 25 µL and 100 µL) with disposable tips
- Distilled water
- ELISA photometer with 450 nm- and 630 nm-filters
- Timer with 60 min. range or higher
- Microplate washer (optional)
- Vortex or similar mixing tools
- Container for the proper handling of waste and samples after use

5 STORAGE AND STABILITY

- Store the kit and components at 2 °C to 8 °C
- Bring to room temperature (20 °C – 26 °C) at least 30 minutes before use. After use put back into the refrigerator. Avoid long time storage at room temperature.
- Do not use the kit or components after the expiry date. For expiry date of the original packed kit see kit label.
- Close the bottles immediately after use.
- Store the plate incl. desiccant in the provided zip-lock pouch. Modules that are not used should always be stored under this condition.
- Ensure that kit components do not freeze.

6 PRECAUTIONS

- Serum and plasma samples should be treated as potentially infectious materials. Wear gloves and proper laboratory attire when handling sample materials. Do not eat, drink or smoke in areas where specimen or kit reagents are handled. Do not pipette with the mouth. In case of skin contact, wash with a germicidal soap and copious amounts of water. Seek medical advice if indicated.
- The PSA standards and controls are of human origin. They have been tested and confirmed negative for HIV, HBsAg and HCV. However, all standards should be treated as potential biohazards.
- Due to the potentially infectious character of samples and kit components all materials that have come in contact with these materials should be sterilized and disposed of according to local regulations. This also includes the liquid waste.
- The assay reagents contain preservatives, TMB, H_2O_2 or sulphuric acid and may be harmful if ingested. A direct skin or mucosa contact should be avoided. In case of skin contact, wash thoroughly with water and seek medical attention if required.
- The stop solution contains H_2SO_4. Since the H_2SO_4 used to terminate the color reaction is corrosive, the instrumentation employed to dispense it should be thoroughly cleaned after use.
- Do not interchange reagents from different LOT# or different suppliers.
- Avoid reagent or sample carry-over by using fresh tips for solutions and samples.
- Do not use test kit if zip lock pouch or bottles have been damaged.

7 GUIDELINE FOR SAMPLE COLLECTION; PREPARATION AND STORAGE

Serum or plasma (EDTA, lithium heparin or citrate) can be used in this assay.

Note: Samples containing sodium azide should not be used in the assay.
In general, it should be avoided to use hemolytic, icteric or lipemic specimens. For further information, refer to chapter "Interfering substances".

Important notes before blood drawing for PSA determination:
As different factors could influence the PSA level in blood, so if one is using patient samples, make sure the patient has avoided the following conditions before taking the blood samples:

The following conditions may lead to an increase of PSA levels:
- Manipulation of the prostate during medical examinations like digital rectal examination (DRE), transrectal prostatic ultrasound, etc.
- Prostatitis
- Biking
- Sexual intercourse (ejaculation)
- Liver dysfunction

The following conditions may lead to a decreased of PSA levels:
- Intake of 5-alpha-reductase-inhibitors, antiandrogens, or GnRH analoga

7.1 Sample collection and preparation

Serum or plasma should be prepared as soon as possible to avoid hemolysis and to improve the stability of PSA.

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anticoagulant (e.g. Sarsted Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

7.2 Storage of samples

For the assay either fresh serum or plasma samples can be used.
If not used immediately they can be stored at 2-8°C for 1 week. In case of longer storage, freeze at -20°C for up to 12 months. A repeated freezing and thawing of samples should be avoided. Thawed samples should be inverted several times prior to testing.

Note
- Highly lipemic or hemolytic samples can give incorrect analytical results.
- Samples must be free of microbial contaminations.

– Samples containing high titers of rheumatoid factor and human anti-mouse antibodies (HAMA) could give erroneous results.

7.3 Dilution of samples

If in an initial assay, a specimen is found to contain more analyte than the highest standard, the specimen can be diluted with Zero Standard and re-assayed.
For the calculation of the concentration this dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10 µl sample + 90 µl Zero Standard (mix thoroughly)

b) dilution 1:100: 10 µl dilution a) 1: 10 + 90 µl Zero Standard (mix thoroughly)

8 ASSAY PROCEDURE

Note: It is highly recommended to perform all measurements as duplicates. An independent standard curve should be made for each series of measurements. For best results it is important that the solutions are always added to the wells in the same order to minimize variations in incubation times.

1. Prior to use bring all reagents, standards, controls, and samples to room temperature (18 °C - 25 °C).
2. Check that all components are not expired and take care that bottles and plate (inclusive pouch) are not damaged.
3. Format the required microplate wells. Keep in mind that all measurements should per performed as duplicate. Document position of wells and respective samples, standards and controls to ensure later identification. Put any unused microwell modules back into the zip lock bag with the desiccant, seal bag and store at 2 °C - 8 °C.
4. Pipette 25 µL of standards, controls or samples into each well. Samples with an expected PSA value higher than 25 ng/mL should be diluted with the sample diluent.
5. Incubate 5 min at room temperature.
6. Add 100 µL of Enzyme conjugate into each well.
7. Mix by moving plate on the table (10sec).
8. Incubate 1 h at room temperature.
9. Rinse the wells 5 times with 400 µl distilled water per well, if a plate washer is used
   or
   Briskly shake out the contents of the wells.
   Rinse the wells 5 times with 300 µl distilled water per for manual washing. Strike the sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
10. Add 100 µL Substrate solution into each well.
11. Incubate 20 min at room temperature.
12. Stop the enzymatic reaction by adding 100 µL/well stop solution.
13. Read absorbencies (OD) at 450 nm and at at 620 to 630 nm (background subtraction, recommended) with a microtiter plate reader.
    It is recommended that the wells be read within 10 minutes after adding the Stop solution.

8.1 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and specimen samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 25 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

8.1.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Zero Standard 0 (0 ng/mL)	0.05
Standard 1 (1.56 ng/mL)	0.24
Standard 2 (3.12 ng/mL)	0.39
Standard 3 (6.25 ng/mL)	0.74
Standard 4 (12.5 ng/mL)	1.27
Standard 5 (25.0 ng/mL)	2.01

9 ASSAY CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0.2 ng/ml and 25 ng/ml.

9.2 Specificity of Antibodies (Cross-Reactivity)

The following substances were tested for cross-reactivity of the assay. No interference with the assay was found for:

Substance	Amount added
APF	10 µg/ml
CEA	10 µg/ml
HCG	10 µg/ml
Lactalbumin	10 µg/ml

10 QUALITY CONTROL

It is recommended that internal controls are used in every assay in duplicate. Control results should be within established ranges and should preferably represent low, medium, and high concentrations.

11 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

11.1 Interfering Substances

Hemoglobin (up to 0.1 mg/ml), Bilirubin (up to 0.2 mg/ml) and Triglyceride (up to 15 mg/ml) have no influence on the assay results.

11.2 Drug Interferences

The following cytostatic drugs were tested and no interference with the assay was found:

Drug	Concentration tested (µg/ml)
Carboplatin	700
Cisplatin	200
Calcium Folinate	2.3
Cyclophosphamide	1000
5-Fluorouracil	500
Doxorubin HCl	72
Dexamethasone	11
Diethylstilbestrol	12
Flutamide	10
Methotrexate	50

The following hypertension drugs were tested and no interference with the assay was found:

Drug	Concentration tested (µg/ml)
Simvastatin	0.1
Irbesartan	1.5
Sildenafil Citrate	5
Furosemide	200

The following antimicrobial agent was tested and no interference with the assay was found:

Compound	Concentration tested (%)
Benzalkonium Chloride	0.5

12 LEGAL ASPECTS

12.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

12.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results are invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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