DRG® PSA ELISA (EIA-4105)

Revised 22 Feb. 2011 rm (Vers. 2.1) **USA: RUO**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

For the Quantitative Determination of Total Prostate Specific Antigen **(tPSA)** Concentration in Human Serum by a Microplate immunoenzymometric assay.

PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti- PSA antibody.
Upon mixing monoclonal biotinylated antibody, the enzymelabeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$Enz\,Ab_{(p)} + Ag_{PSA} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Enz\,Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ =Biotinyiated Antibody (Excess Quantity)
Ag_{PSA} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(P)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(p)}$-Ag_{PSA}-$^{Btn}Ab_{(m)}$ =Antigen-Antibodies Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation
Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinyiated antibody. This interaction is illustrated below:

$^{Enz}Ab_{(p)}$-Ag_{PSA}-$^{Btn}Ab_{(m)}$+$Streptavidin_{c.w}$→Immobilized complex
$Streptavidin_{c..w.}$= Streptavidin immobilized on well
Immobilized complex = complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of

known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

REAGENTS
Materials Provided:

A. Prostrate Specific antigen (PSA) 1 ml/vial - Icons A-F
Six (6) vials of references PSA Antigen at levels of 0**(A),** 5**(B),** 10(**C**), 25**(D),** 50**(E)** and 100**(F)** ng/ml. Store at 2-8°C. A preservative has been added.

Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the 1st IS 96/670.

B. PSA Enzyme Reagent - 13ml/vial
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate - 96 wells
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate - 20 ml
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.

E. Substrate A - 7ml/vial
One (1) bottle containing tetramethylbenzidine (TMB) *in* buffer. Store at 2-8°C.

F. Substrate B - 7ml/vial
One (1) bottle containing hydrogen peroxide (H_20_2) in buffer. Store at 2-8°C.

G. Stop Solution - 8ml/vial
One (1) bottle containing a strong acid (1N HCl). Store at 2-30°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C.
Note 3: Above reagents are for a single 96-well microplate

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Required But Not Provided:

1. Pipette(s) capable of delivering 25 & 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

PRECAUTIONS

For Research Use Only

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.

2. **Working Substrate Solution**
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification.
   Mix and label accordingly. Store at 2 - 8°C.

Note: Do not use the working substrate if it looks blue.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).

1. Format the microplates' wells for each serum reference, control and specimen to be assayed in duplicate.
   Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.100 ml (100µl) of the PSA Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate 30 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.

7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**

8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

   DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**

11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of PSA in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding PSA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of PSA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.142) intersects the dose response curve at (23.6 ng/ml) PSA concentration (See Figure 1).

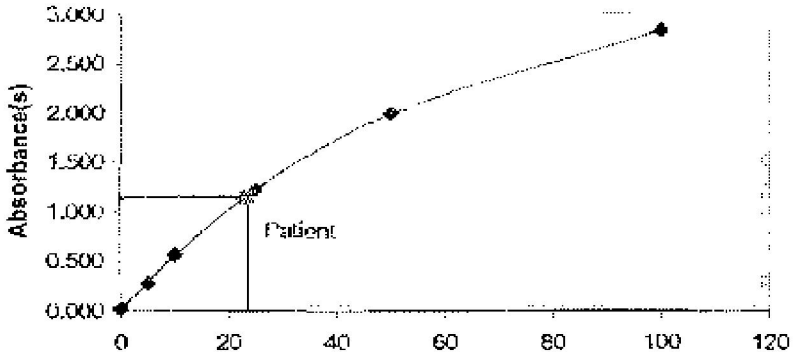
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.019		
	B1	0.019	0.019	0
Cal B	C1	0.279		
	D1	0.273	0.276	5
Cal C	E1	0.567		
	F1	0.559	0.563	10
Cal D	G1	1.248		
	H1	1.179	1.213	25
Cal E	A2	2.051		
	B2	1.947	1.999	50
Cal F	C2	2.892		
	D2	2.775	2.833	100
Sample	E3	1.186		
	F3	1.099	1.142	23.6

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



PSA Values in ng/ml

A. Assay Performance

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
3. Highly lipemic or hemolyzed specimen(s) should similarly not be used.
4. If more than (1) plate is used, kit is recommended to repeat the dose response curve.
5. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Specimens with PSA concentrations above 100 ng/ml may be diluted (for example 1/10 or higher) with normal female serum (PSA = 0 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from these instructions may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

12. It is important to calibrate all the equipment e.g., pipettes, readers, washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC – for this and other devices, may be requested from the supplier.

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