Monobind Inc.
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AccuLite
CLIA Microwells

Total Prostate Specific Antigen (PSA) Test System

Product Code: 2175-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Prostate Specific Antigen (PSA) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Chemiluminescence

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prostate Specific Antigen (PSA) is a serine protease with chymotrypsin-like activity.[1,2] The protein is a single chain glycoprotein with a molecular weight of 28.4 kDA.[3] PSA derives its name from the observation that it is a normal antigen of the prostate, but is not found in any other normal or malignant tissue.

PSA is found in benign, malignant and metastatic prostate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found to be more useful than prostatic acid phosphatase (PAP) in the diagnosis and management of patients due to increased sensitivity and specificity.[4]

In this method, PSA calibrator, patient specimen or control is first added to a streptavidin-coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of PSA are added and the reactants mixed. Reaction between the various PSA antibodies and native PSA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-PSA antibody bound conjugate is separated from the unbound enzyme-PSA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce light (*luminescence)*.

The employment of several serum references of known total prostate specific antigen (PSA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with PSA concentration.

3.0 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 enzyme-labeled 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:

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

$^{Btn}Ab_{(m)}$ =Biotinylated Antibody (Excess Quantity)
Ag_{PSA} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$ =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 biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$+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, determined by reaction with a substrate that generates light, 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.

4.0 REAGENTS

Materials Provided:

A. PSA Calibrators – 1 ml/vial – Icons A-F
Six (6) vials of serum references PSA Antigen at levels of 0(**A**), 5(**B**), 10(**C**), 25(**D**), 50(**E**) and 100(**F**) ng/ml. A preservative has been added. Store at 2-8°C.
The material is calibrated against the WHO (National Institute for Biological Standards and Controls) IS# 96/670.

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

C. Light Reaction Wells – 96 wells – Icon ⇓
One 96-well white 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/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C (see Reagent Preparation Section).

E. Signal Reagent A – 7 ml/vial – Icon C^{A}
One (1) vial containing luminol in buffer. Store at 2-8°C (see Reagent Preparation Section).

F. Signal Reagent B – 7 ml/vial – Icon C^{B}
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C (see Reagent Preparation Section).

G. Product Insert

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.
Note 3: Above reagents are for a 96-well microplate. For other kit configurations, refer to the table at the end of this insert.

4.1 Materials Required But Not Provided:
1. Pipette(s) capable of delivering 0.025 & 0.100 ml (25 &100 µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350 ml (100 & 350 µl) volumes with a precision of better than 1.5% (optional).
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Luminometer.
5. Container(s) for mixing of reagents (see below).
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Storage container for storage of wash buffer.
11. Distilled or deionized water.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
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 required tests. 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

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 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 samples should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8ºC for a maximum period of five (5) days. If the specimen(s) can not be assayed within this time, the sample(s) may be stored at temperatures of -20ºC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050 ml (50 µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C.
2. **Working Signal Reagent Solution -** Store at 2 - 8°C.
   Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1ml of B per two (2) eight well strips. (A slight excess of solution is made.) **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

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

*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient 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 (25 µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100 µl) of the PSA Tracer 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 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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 four (4) additional times.**
8. Add 0.100 ml (100 µl) of working signal reagent 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 PLATE AFTER SIGNAL ADDITION
9. Incubate at room temperature for five (5) minutes in the dark.
10. Read the Relative Light Units (RLUs) in each well, for 0.2 – 1.0 seconds/ well, using a microplate luminometer. **The results should be read within thirty (30) minutes of adding the signal reagent solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of PSA in unknown specimens.
1. Record the RLUs obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLUs 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 RLUs 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 RLUs (17210) of the unknown intersects the calibration curve at 12.3 ng/ml PSA concentration (See Figure 1)*.

Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

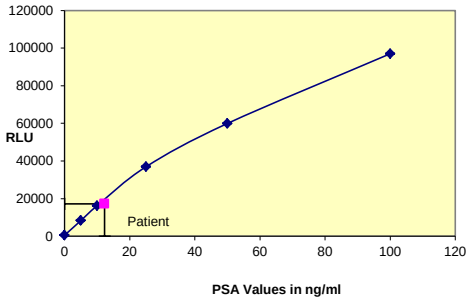
EXAMPLE 1

Sample I.D.	Well Number	RLUs (A)	Mean RLUs (B)	Value (ng/ml)
Cal A	A1	520	530	0
	B1	541		
Cal B	C1	8466	8391	5
	D1	8317		
Cal C	E1	16404	16252	10
	F1	16100		
Cal D	G1	36760	36933	25
	H1	37106		
Cal E	A2	59233	59957	50
	B2	60680		
Cal F	C2	97360	100000	100
	D2	102640		
Patient	E2	17450	17210	12.3

	F2	16970		

* 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. In addition, the RLUs of the calibrators have been normalized to 100,000 RLUs for the F calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

Figure 1



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11.0 QC PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
9. 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.
10. 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.
11. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for AccuLite® CLIA procedure have been formulated to eliminate maximal interference; however, potential interactions between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato, LM, Stuart, MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin. Chem.* 1988: 3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. PSA is elevated in benign prostatic hyperplasia (BPH). Clinically, an elevated **PSA value alone is not of diagnostic value as a specific test for cance**r and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures such as prostate biopsy and DRE (*Digital Rectal Examination*) report. Free PSA determinations may be helpful in regard to the differential diagnosis of BPH and prostate cancer conditions.[5]
8. Due to the variation in the calibration used in PSA/ fPSA test kits and differences in epitopic recognition of different antibodies it is always suggested that the patient sample should be tested with PSA/ fPSA tests made by the same manufacturer. (***Monobind Inc. offers a fPSA Chemiluminescence test that should be used for consistency reasons, when needed.***)

13.0 EXPECTED RANGES OF VALUES

Healthy males are expected to have values below 4 ng/ml.[4]

TABLE I
Expected Values for the PSA AccuLite® CLIA Test System

Healthy Males	< 4 ng/ml

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal"-persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of PSA AccuLite® CLIA test were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Tables 2 & Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	0.55	0.03	5.5%
Level 2	20	3.84	0.22	5.7%
Level 3	20	25.21	0.86	3.4%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	10	0.51	0.06	11.8%
Level 2	10	3.92	0.32	8.1%
Level 3	10	25.01	1.07	4.3%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The sensitivity of PSA AccuLite® CLIA test system is defined as the smallest single value which can be distinguished at 2 S.D. from a mean of twenty (20) replicates of '0' calibrator. This method has a sensitivity of 0.006ng/ml.

14.3 Accuracy

The PSA AccuLite® CLIA test system was compared with a reference method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 180. The least square regression equation and the correlation coefficient were computed for the PSA AccuLite® CLIA test system in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
This Method (X)	6.18	y = -0.0748+0.986(X)	0.991
Reference (Y)	6.45		

15.0 REFERENCES

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6. Prestigiacomo AF, Stamey TA, 'Physiological variations of serum prostate antigen in the (4-10 ng/ml) range in male volunteers.' *J. Urol*, 155, 1977-80 (1996).
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MP2175 Product Code: 2175-*300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)

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IVD 2° 8° CE 0050
EC REP CEpartner4U, Esdoornlaan 13 3951 DBMaarn, The Neatherlands www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2° 8° Temperature Limitation Storage Condition (2-8°C)	Consult Instructions for Use
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP Authorized Rep in European Country	CE 0050 European Conformity	