
PROSTATE SPECIFIC ANTIGEN (PSA) CHEMILUMINESCENCE

IMMUNOASSAY KIT

Catalog No. CL0206-2

INTENDED USE

The Autobio prostate specific antigen (PSA) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of PSA concentration in human serum.

INTRODUCTION

Prostate Specific Antigen (PSA), a glycoprotein with a molecular weight of 34,000D, was first isolated by Wang *et. Al.* in 1979¹. PSA is a kallikrein-like serine protease that is produced exclusively by the epithelial cells of the prostate. 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. 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. 30-50% of patients with benign prostatic hyperplasia have elevated serum PSA concentrations, depending on the size of the prostate and the degree of obstruction, and the concentrations are increased in 25~92% of patients with prostate cancer, depending on tumour volume^{2, 3, 4}. Elevated levels have not been reported for cancers of the lung, breast, colon, rectum, stomach, pancreas or thyroid^{1, 5, 6}.

Digital rectal examination, cystoscopic examination and prostate biopsy all can cause elevations of the serum PSA concentration². Conditions such as bacterial prostatitis and acute urinary retention also can increase the serum PSA level^{7, 8, 9, 10}.

Recent studies also indicate that PSA measurements can enhance early prostate cancer detection when combined with digital rectal examination (DRE). When compared to prostatic acid phosphatase (PAP), PSA is a more precise and useful marker in all clinical situations^{11, 12, 13}.

PRINCIPLE OF THE TEST

The PSA CLIA kit is based on a solid phase sandwich enzyme-linked immunosorbent assay. The assay system utilizes an anti-PSA monoclonal antibody for solid phase (microtiter wells) immobilization and another anti-PSA monoclonal antibody as antibody-enzyme (horseradish peroxidase) conjugate reagent. PSA in the reference standards or in the patient's specimens binds to anti-PSA MAB on the well and the anti-PSA second antibody then binds to PSA. Unbound protein and HRP conjugate are removed by washing. Upon the addition of the substrate, the horseradish peroxidase activity bound on the wells is then assayed by a chemiluminescence reaction. The related light unit (RLU) of the reaction is proportional to the concentration of PSA presented in the specimen.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to prostate specific antigen (anti-PSA MAb) (1 plate, 48 wells/96wells)
2. Enzyme Conjugate Reagent: Horseradish Peroxidase (HRP) labeled anti-PSA MAb in Stabilizing Buffer (1 vial, 6.0ml/11.0 ml)
3. Reference Standards: 0, 2, 4, 15, 50, and 100ng/ml PSA in Stabilizing Buffer (6 vials, 0.5ml/ea)
4. Substrate A: (1 vial, 3.5ml/6.0ml)
5. Substrate B: (1 vial, 3.5ml/6.0ml)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit.

1. Distilled water
2. Precision pipettes for delivery of 20-200 μ l, 100-1000 μ l (the use of accurate pipettes with disposable plastic tips is recommended)
3. Luminometer
4. Vortex Mixer or equivalent

5. Washer for microplate
6. Quality control specimens
7. Incubator
8. Absorbent paper

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2~8°C upon receipt. The test kit may be used throughout the expiration date of the kit (6 months from the date of manufacture). Refer to the package label for the expiration date.
2. Reconstituted standards should be used within 14 days and be frozen at -20°C for long term storage. Repeated freezing and thawing of the standards should be avoided. Microplate after first use should be kept in a sealed bag with desiccants to minimize exposure to damp air. Other opened components will remain stable for at least 2 months, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Serum is the recommended sample type for this assay. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and stored up to 48 hours at 2~8°C. For longer storage, freeze the specimens at -20°C. Thawed samples must be mixed prior to testing.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The reference standards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the standards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18~25°C) prior to use.
2. Reconstitute each lyophilized standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 10 minutes. Reconstituted standards should be stored sealed at 2~8°C.

IMPORTANT NOTES

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and invalid results.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 25µl of PSA standards, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is important to have complete mixing in this step.
5. Incubate at 37°C for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter plate 5 times with distilled water. Strike the plate sharply onto absorbent paper to remove residual water droplets. The volume of the well is about 300µl.

7. Dispense 50µl of Substrate A, then 50µl of Substrate B into each well. Gently mix for 10 seconds.
8. Put the microplate into the detecting chamber of Luminometer for 5 minutes, then read the RLU values of each well.

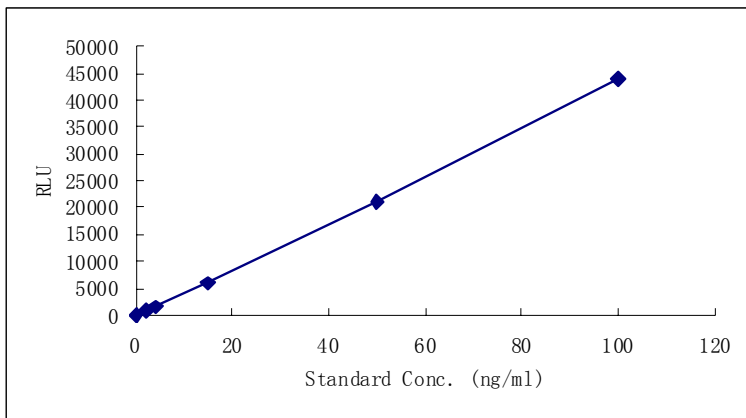
CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. On linear graph paper plot the RLU (ordinate) obtained from each reference standard against the corresponding concentration of PSA in ng/ml (abscissa) and draw a calibration curve through the reference standard points by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a point to point function curve fitting is recommended.
5. Any diluted specimens must be corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

PSA (ng/ml)	RLU
0	138.382
2	793.266
4	1607.1
15	5960.29
50	21004.9
100	44025.2



EXPECTED VALUES

Each laboratory should establish its own normal range. Following information is given only for guidance. Approximately 95% of the normal healthy population has PSA levels less than 4ng/ml.

PERFORMANCE

A. Sensitivity

The lower detection limit is calculated from the standard curve by identifying the resulting concentration of the mean RLU of standard diluent (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the Autobio PSA CLIA kit is not higher than 0.5ng/ml.

B. Specificity

No interference was detected with the performance of Autobio PSA CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	Concentration
AFP	500ng/ml
CEA	500ng/ml
SF	400ng/ml

C. Precision

a. Intra-assay Precision

Intra-assay precision was determined by assaying 20 replicates of each control sera.

Serum	Number	Mean	SD	CV (%)
Low titer	20	4.52	0.34	7.52
High titer	20	15.26	0.96	6.29

b. Inter-assay Precision

Inter-assay precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV (%)
Low titer	10	3.95	0.31	7.85
High titer	10	15.29	0.91	5.95

D. High Dose Hook Effect

No hook effect occurred with PSA concentration up to 900ng/ml.

E. Accuracy

For 72 specimens in the range of 0ng/ml to 100ng/ml, the correlation between the Autobio PSA CLIA kit and Roche Elecsys assay was as follows:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation Coefficient
Roche® (ECLIA)	72	$y = 1.046x - 0.9781$	0.943

LIMITATIONS

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert and with adherence to good laboratory practice.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
- For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

	BATCH CODE
	USE BY
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	TEMPERATURE LIMITATION
	CATALOGUE NUMBER



CONSULT INSTRUCTIONS FOR USE

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