IVD

# FREE PROSTATE SPECIFIC ANTIGEN (F-PSA) CHEMILUMINESCENCE IMMUNOASSAY KIT

## Catalog No. CL0208-2

***INTENDED USE***

The Autobio free prostate specific antigen (f-PSA) chemiluminescence immunoassay (CLIA) kit is intended for the quantitative determination of f-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 1979[1]. It is mainly found in the cytoplasm of prostate acinar cells and ductal epithelium[2]. In addition to being present in normal tissue, PSA is also present in prostatic cancerous tissue, benign hyperplastic tissue, in prostatic fluid and seminal plasma, and is therefore a useful clinical marker for prostate cancer [3, 4, 5]. Two immunoreactive forms of PSA are found in serum: free and complexed PSA. The complexed form is given by the binding of alpha-1-antichymotrypsin (ACT) to the active site of PSA [6, 7]. The PSA molecule which is not bound to the serine protease inhibitor ACT, named Free PSA, is found in lower concentrations than the complexed form [6, 7].

Current methods of screening men for prostate cancer utilize the detection of the major PSA-ACT form. Levels of 4.0ng/ml or higher are strong indicators of the possibility of prostatic cancer. However, elevated serum PSA levels have also been attributed to benign prostatic hyperplasia and prostatitis, leading to a large percentage of false positive screening results. A potential solution to this problem involves the determination of free PSA levels. Preliminary studies have suggested that the percentage of free PSA is lower in patients with prostate cancer than those with benign prostatic hyperplasia. Thus, the measurement of free serum PSA in conjunction with total PSA, can improve specificity of prostate cancer screening in selected men with elevated total serum PSA levels, which would subsequently reduce unnecessary prostate biopsies with minimal effects on cancer detection rates.

The proportion, or percent, of free PSA determined by comparing the concentration of free PSA to the concentration of total PSA has been proposed as a way to improve the discrimination between BPH and prostate cancer, especially in those men with intermediate levels of total serum PSA.[8,9-14]

***PRINCIPLE OF THE TEST***

The f-PSA CLIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The f-PSA molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by chemiluminescence reactions. The Related Light Unit (RLU) of the reaction is proportional to the concentration of f-PSA present in the sample.

***MATERIALS PROVIDED***

1. Antibody Coated Microtiter Plate: Microplate coated with monoclonal antibodies to free prostate specific antigen (anti- f-PSA MAB) (1 plate, 48 wells/96wells)
2. Sample Diluent: (1 vial, 6.0ml/11.0ml)
3. Enzyme Conjugate Reagent: horseradish peroxidase (HRP) labeled anti-PSA MAB in Stabilizing Buffer (1 vial, 11.0ml/22.0ml)
4. Reference Standards: 0, 0.1, 0.5, 2.0, 5.0, and 10.0 ng/ml f-PSA in Stabilizing Buffer (6 vials, 0.5ml/ea)
5. Substrate A: (1 vial, 3.5ml/6.0ml)
6. 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 20-200µl, 100-1000µl (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℃ 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. 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℃. For longer storage, freeze the specimens at -20℃. 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 satandards 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 satandards 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℃) 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℃.

***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. Dispense 100μl of f-PSA standards, specimens, and controls into appropriate wells.
2. Dispense 100μl of sample diluent to each well. Mix gently for 30 seconds.
3. Incubate at 37°C for 60 minutes.
4. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with distilled water. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets. The volume of the well is about 300μl.
5. Dispense 200μl of enzyme conjugate reagent into each well. Mix well.

6. Incubate at 37°C for another 60 minutes.
7. At the end of the 60 minute incubation, remove the contents and wash the wells as described in step 4 above.
8. Dispense 50μl of Substrate A, then 50μl of Substrate B into each well. Gently mix for 10 seconds.
9. Put the microplate into the detecting chamber of Luminometer for 5 minutes, then read the RLU values of each well.

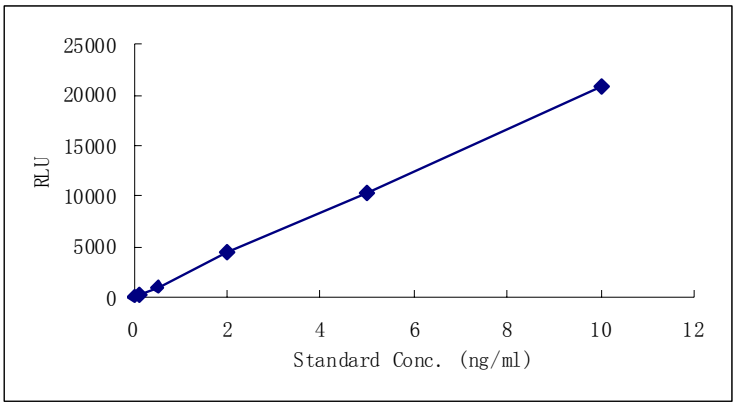
***CALCULATION OF RESULTS***

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 f-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.

| f-PSA (ng/ml) | RLU |
|---|---|
| 0 | 155.135 |
| 0.1 | 348.627 |
| 0.5 | 1091.61 |
| 2 | 4466.71 |
| 5 | 10300.5 |
| 10 | 20920.7 |




***PERFORMANCE***

***A. Sensitivity***

The lower detection limit is calculated from the standard curve by identifying the concentration corresponding to the mean RLU of standard diluent (based on 10 replicate analyses) plus 2 SD. In this way, the sensitivity of the Autobio f-PSA CLIA kit is not higher than 0.05ng/ml.

***B. Specificity***

No interference was detected with the performance of Autobio f-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 | 0.56 | 0.05 | 8.93 |
| High titer | 20 | 7.26 | 0.53 | 7.30 |

***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 | 0.52 | 0.04 | 7.69 |
| High titer | 10 | 7.63 | 0.51 | 6.68 |

***D. Accuracy***

For 72 specimens in the range of 0ng/ml to 10ng/ml, the correclation between the Autobio f-PSA CLIA kit and Roche Elecsys assay was as follows:

| **Reference** | **Number of Specimens** | **Least Square Regression Analysis** | **Correlation Coefficient** |
|---|---|---|---|
| Roche® (ECLIA) | 72 | y = 1.022x -0.9124 | 0.944 |

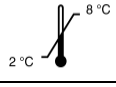
***LIMITATIONS***

1. 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.
2. 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.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. 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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