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REF

9054-16

CHEMILUMINESCENCE
ENZYME IMMUNOASSAY (CLIA)
FREE PROSTATE SPECIFIC ANTIGEN (f-PSA)

Free PSA

REF 9054-16

Enzyme Immunoassay for the Quantitative Determination of free Prostate Specific Antigen (fPSA) in Human Serum

INTRODUCTION OF CHEMILUMINESCENCE IMMUNOASSAY

Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).

ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.

Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.

The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panels, such as T3, T4, TSH, Hormone panels, such as hCG, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISA that have been widely used in many research and diagnostic applications.

Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

The CLIA Kits have been validated on the **MPL1** and **MPL2** microplate luminometers from Berthold Detection System, **Lus2** microplate luminometer from Anthos, **Centro LB960** microplate luminometer from Berthold Technologies, and **Platelumino** from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

INTRODUCTION OF fPSA IMMUNOASSAY

Introduction of the Immunoassay

Human Prostate Specific Antigen (PSA) is a 33 kD serine proteinase which, in human serum, is predominantly bound to alpha 1-antichymotrypsin (PSA-ACT) and alpha 2-macroglobulin (PSA-AMG). Trace amounts of alpha 1-antitrypsin and inter-alpha trypsin inhibitor bound to PSA can also be found. Any remaining PSA is in the free form (f-PSA).¹⁻³ 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.⁴ 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.⁵ A potential solution to this problem involves the determination of free PSA levels.¹⁻³ 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.⁶

TEST PRINCIPLE

The Free PSA ELISA test is a solid phase, two-site immunoassay. One antibody is coated on the surface of the microtiter wells and another antibody labeled with horseradish peroxidase is used as the tracer. The 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 adding the substrate reagents and undergoing the chemiluminescent reactions. The intensity of the emitting light from the associated well is proportional to the amount of enzyme present and is directly related to the amount of PSA antigen in the sample. By reference to a series of PSA standards assayed in the same way, the concentration of PSA in the unknown sample is quantified.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

1. Anti-fPSA antibody coated 96 well microtiter plate.
2. Sample Diluent, 12 ml.
3. Enzyme conjugate reagent, 22 ml.
4. fPSA reference standards set, contains 0, 0.1, 0.5, 2.0, 5.0 and 10.0 ng/ml, liquid, Ready for use.
5. 50x Wash Buffer Concentrate, 15 ml
6. Chemiluminescence Reagent A, 6.0 ml
7. Chemiluminescence Reagent B, 6.0 ml

Materials required but not provided:

1. Distilled water.
2. Precision pipettes: 0.05ml, 0.1ml, 0.2ml
3. Disposable pipette tips.

4. Glass tube or flasks to mix Reagent A and B.
5. Microtiter well reader.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

SPECIMEN COLLECTON & PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use, and mixed by gently inverting or swirling prior to use. Do NOT induce foaming.
2. To prepare substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **100**µL of fPSA standards, specimens, and controls into appropriate wells.
2. Dispense **100**µL 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.
5. Rinse and flick the microtiter wells 5 times with washing buffer(1X).
6. Strike the wells sharply onto absorbent paper to remove residual water droplets.
7. Dispense **200**µL of enzyme conjugate reagent into each well. Mix well.
8. Incubate at 37°C for 60 minutes.
9. Remove the contents and wash the plate as described in step 4, 5, and 6 above.
10. Dispense **100**µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
11. Read wells with a chemiluminescence microwell reader 5 minutes later. (Between 5 and 20 min. after dispensing the substrates).

Important Note:

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. If there are bubbles existing in the wells, the false readings will be created. Please use distilled water to remove the bubbles before adding the substrate.

CALCULATION OF RESULTS

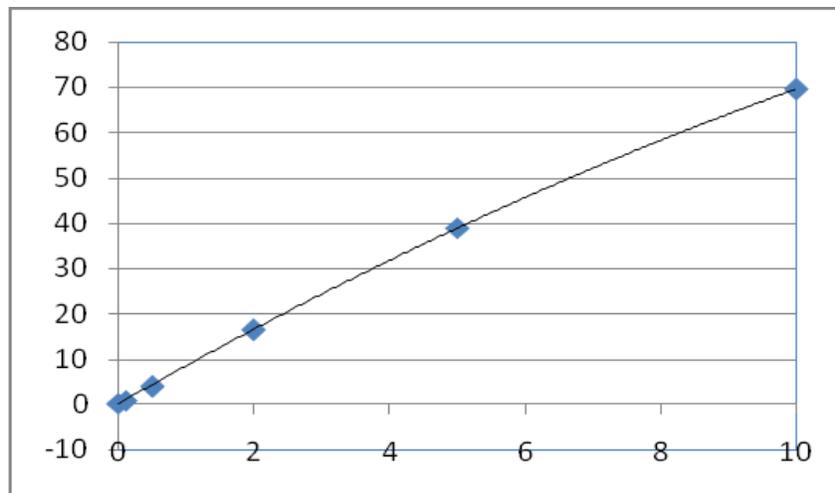
1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend using proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regression or cubic spline regression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against fPSA concentration in ng/ml on linear graph paper, with RLU on the vertical (y) axis and concentration on the horizontal (x) axis.

- Using the mean absorbance value for each sample, determine the corresponding concentration of fPSA in ng/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required to run assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

fPSA (ng/ml)	Relative Light Units (RLU) (10 ⁴)
0.0	0.16
0.1	1.19
0.5	3.91
2.0	16.4
5.0	38.9
10.0	69.6



EXPECTED VALUES AND SENSITIVITY

For total PSA levels between **3.0 and 4.0 ng/ml**, using a **19%** cutoff point for percent free-PSA would result in detection of 90% of all cancers.

For total PSA levels between **4.1 and 10.0 ng/ml**, the most appropriate cutoff point for free-PSA is **24%**. At this cutoff point, 95% of the cancers would be detected.

Free PSA **>27%** with lower likelihood of [prostate cancer](#).

The minimum detectable concentration of f-PSA in this assay is estimated to be 0.05 ng/ml.

STORAGE

- Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.

2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

LIMITATIONS

There are some limitations of the assay.

- 1) As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
- 2) Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects.
- 3) The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background reading.

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