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PROSTATE SPECIFIC ANTIGEN (PSA)

REF

Catalog No. C29-336

IVD

For In Vitro Diagnostic Use Only

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

INTRODUCTION

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.

Immunospec 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.

INTRODUCTION OF PSA IMMUNOASSAY

Human prostate-specific antigen (PSA) is a serine protease, a single chain glycoprotein with a molecular weight of approximately 34,000 daltons containing 7% carbohydrate by weight. 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. PSA is not present in any other normal tissue obtained from men, nor is it produced by cancers of the breast, lung, colon, rectum, stomach, pancreas or thyroid. Besides, it is functionally and immunologically different from prostatic acid phosphatase (PAP). Elevated serum PSA concentrations have been reported in patients with prostate cancer, benign prostatic hypertrophy, or inflammatory conditions of other adjacent genitourinary tissues,

but not in apparently healthy men, men with non-prostatic carcinoma, apparently healthy women, or women with cancer. Reports have suggested that serum PSA is one of the most useful tumor markers in oncology. 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. Recent studies also indicate that PSA measurements can enhance early prostate cancer detection when combined with digital rectal examination (DRE).

TEST PRINCIPLE

Immunospec PSA CLIA 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-PSA antibody coated 96 well microtiter plate.
2. Assay buffer; 12 ml.
3. Enzyme conjugate reagent, 12 ml.
4. PSA reference standards set, contains 0, 2, 4, 15, 50, and 100 ng/ml PSA, liquid, Ready for use.
5. 20x Wash Buffer, 30 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.

REAGENT PREPARATION

1. To prepare substrate solution, make an 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
2. Prepare the washing solution by diluting 1 part of the 20X PBS concentrate to 19 parts of distilled water.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 50µL of PSA standards, specimens, and controls into appropriate wells.
2. Dispense 100µL of Assay Buffer to each well. Mix gently for 30 seconds.
3. Incubate at room temperature for 45 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.
6. Strike the wells sharply onto absorbent paper to remove residual water droplets.
8. Dispense 100µL of enzyme conjugate reagent into each well. Mix well.
9. Incubate at room temperature for 60 minutes.

- Remove the contents and wash the plate as described in step 4,5, and 6 above.
- Dispense **100**µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
- Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

Important Note:

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

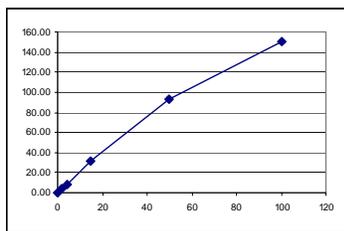
CALCULATION OF RESULTS

- Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
- We recommend to use a 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 PSA 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 PSA 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 that running 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.

PSA (ng/ml)	Relative Light Units (RLU) (10 ⁴)
0	0.24
2	4.00
4	8.23
15	31.65
50	93.50
100	150.03



EXPECTED VALUES AND SENSITIVITY

Healthy males are expected to have PSA values below 4 ng/ml. The minimum detectable concentration of PSA in this assay is estimated to be 0.5 ng/ml.

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