DRG® PSA, Free (EIA-1792)

Revised 24 Jan. 2011 rm (Vers. 4.1) **RUO**

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

THIS KIT IS INTENDED FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRINCIPLE OF THE TEST

The f-PSA ELISA test is a solid phase two-site immunoassay. An anti-f-PSA monoclonal antibody is coated on the surface of the microtiter wells and a rabbit anti-PSA antibody labeled with horseradish peroxidase is used as the tracer. The f-PSA molecules present in the standard solution or sera are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen- antibody-enzyme complex, the unbound antibody-enzyme tracers are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by a colorimetric reaction. The intensity of the color formed is proportional to the concentration of f-PSA present in the sample.

REAGENTS

Materials provided with the kits:

- Murine Monoclonal Anti-Free PSA coated microtiter plate with 96 wells.
- Reference standards containing 0, 1.0, 2.5, 5.0, 10.0, and 25.0 ng/ml f-PSA, 1 ml/vial, 1 set.
- f-PSA Zero Buffer, 13 ml.
- Enzyme Conjugate Reagent, 22 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided

- Distilled water.
- Precision pipettes: 5 µl, 100 µl, 200 µl, and 1.0 ml.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Microtiter plate shaker
- Graph paper
- A Microtiter plate reader with a bandwidth of 10nm or less and an optical density range 0.2 OD or greater at 450 nm.

STORAGE OF TEST KIT AND INSTRUMENTATION

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. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8 °C.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense **50 µl** of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl of f-PSA Zero Buffer into each well.
4. Gently mix for 30 seconds.
5. Incubate at room temperature (18-25°C) for 60 minutes.
6. Remove the incubation mixture by emptying plate contents into a suitable waste container.
7. Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 200 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 60 minutes.
11. Remove the incubation mixture by emptying plate contents into a suitable waste container.
12. Rinse and empty the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
13. Strike the wells sharply onto absorbent paper to remove residual water droplets.
14. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
15. Incubate at room temperature for 20 minutes.
16. Stop the reaction by adding 100 µl of Stop Solution to each well.
17. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
18. Using a microtiter plate reader, read the optical density at 450 nm ***within 15 minutes***.

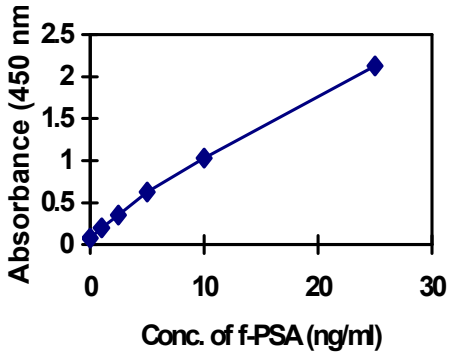
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards, control, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of f-PSA in ng/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against f-PSA concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

f-PSA (ng/ml)	Absorbance (450 nm)
0	0.047
1.0	0.244
2.5	0.527
5.0	0.919
10.0	1.546
25.0	2.837



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