

### IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards & samples is not mandatory but may provide information on reproducibility & application errors.












### 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). The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result. (3). Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits. (4). Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

### BIBLIOGRAPHY

(1). Christensson A, Bjork T, Nilsson O, et al. Serum Prostate Specific Antigen Complexed to 1-Antichymotrypsin As An Indicator of Prostate Cancer. *J. of Urol.* 150:100-105; 1993. (2). Lilja H, Christensson A, Dahlen U, et al. Prostate-specific antigen in serum occurs predominantly in complex with -1-antichymotrypsin. *Clin Chem.* 1991;37:1618-1625. (3). Stenman U-H, Leinonen J, Alfthan H, Rannikko S, Tuukkanen K, Alfthan O. A complex between prostate-specific antigen and -1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostate cancer: assay of the complex improves clinical sensitivity for cancer. *Cancer Res.* 1991;51:222-226. (4). Catalona WJ, Smith DS, Ratliff TL, Basler JW. Detection of organ-confined prostate cancer is increased through prostate-specific antigen-based screening. *JAMA.* 1993;270:948-954. (5). Stamey TA, Yang N Hay AR, McNeal JE, Freiha, FS, Redwine E. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. *N Engl J Med.* 1987;317:909-916. (6). Catalona et al. Percentage of Free Serum PSA and Prostate Cancer Detection. *JAMA.* 1995;274, No. 15: 1214-1220. (7). Catalona WJ, Smith DS, Ratliff TL, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. *N Engl J Med.* 1991;324:1156-1161. Erratum: *N Engl J Med.* 1991;325:1324. (8). Carter HB, Pearson JD, Metter J, et al. Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. *JAMA.* 1992;267:2215-2220. (9). Smith DS, Catalona WJ. Rate of change in serum prostate-specific antigen levels as a method for prostate cancer detection. *J Urol.* 1994;152:1163-1167. (10). Benson MC, Whang IS, Pantuck A, et al. Prostate specific antigen density: a means of distinguishing benign prostatic hypertrophy and prostate cancer. *J Urol.* 1992; 147:815-816. (11). Catalona WJ, Hudson MA, Scardino PT, et al. Selection of optimal prostate specific antigen cut-offs for early detection of prostate cancer: receiver operating characteristic curves. *J Urol.* 1994;152:2037-2042. (12). Smith DS, Catalona WJ. The nature of prostate cancer detected through prostate specific antigen based screening. *J Urol.* 1994;152:1732-1736. (13). Oesterling JE, Jacobsen SJ, Chute CG, et al: Serum prostate-specific antigen in a community-based population of healthy men. *JAMA* 1993 Aug 18;270:860-864. (14). Catalona WJ, Smith DS, Wolfert RL, et al: Evaluation of percentage of free serum prostate-specific antigen to improve specificity of prostate cancer screening. *JAMA* 1995;274(15);214-1220. (15). Jacobsen SJ, Bergstralh EJ, Guess HA, et al: Predictive properties of serum prostate-specific antigen testing in a community-based setting. *Arch Intern Med* 1996;156:2462-2468. (16). Oesterling JE, Jacobsen SJ, Klee GG, et al: Free, complexed and total serum prostate specific antigen: the establishment of appropriate reference ranges for their concentrations and ratios. *J Urol* 1995;154:1090-1095. (17). Dworschack RT, Thiel RP, Picolli SP: Clinical evaluation of the free/total PSA ratio generated with the Elecsys total and free PSA assays on the Elecsys 1010 and 2010 systems. *Clin Chem* 2001 Jun; 47 (6 Suppl S):A149 (Abstract).

### SYMBOL KEYS

|  |  |   |   |
|--|--|---|---|
|  Temperature Limitation |  Consult Instructions for use       |  Date of Manufacture |  Batch Number / Lot Number         |
|  Manufacturer           |  In vitro Diagnostic Medical Device |  This side up        |  Contains sufficient for <-> tests |
|  Use by                 |  Catalogue Number                   |  Do not reuse        |   |



Manufactured by:

**Zephyr Biomedicals**

A Division of Tulip Diagnostics (P) Ltd.

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0322VER-01



## Enzyme Linked Immunosorbent assay for the Quantitative Determination of Free Prostate Specific Antigen (fPSA) in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

### INTENDED USE

**Qualisa<sup>TM</sup> fPSA** Sandwich ELISA test is intended for the quantitative determination of Free Prostate Specific Antigen (fPSA) in human serum. For In Vitro Diagnostic Use only.

### INTRODUCTION

Human Prostate Specific Antigen (PSA) is a 33KD 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). Current methods of screening men for prostate cancer utilize the detection of the major PSA-ACT form. Levels of 4.0 ng/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.

### PRINCIPLE

**Qualisa<sup>TM</sup> fPSA** Quantitative Test Kit is a sandwich-based enzyme-linked immunosorbent assay. An anti-fPSA monoclonal antibody is coated on the surface of the microtiter wells and another anti-PSA monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The fPSA 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 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 fPSA present in the sample.

### MATERIALS AND COMPONENTS

#### Materials provided with the test kits:

- Coated Microwells: Microwells coated with monoclonal anti- fPSA antibody.
- Sample Diluent. Ready to use
- Enzyme Conjugate. Ready to use.
- TMB Substrate. Ready to use
- Stop Solution. Ready to use
- fPSA Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer viral label.
- Wash Buffer Concentrate (20X).

#### Materials required but not provided

- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

### STORAGE AND STABILITY

1. **Qualisa<sup>TM</sup> fPSA** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to pink at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable upto one week when stored at 2-8°C.

### SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.

4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

#### PRECAUTIONS

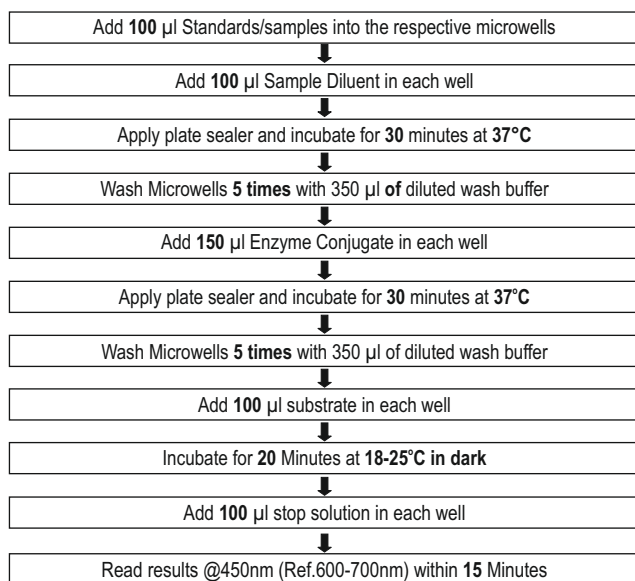
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

#### REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water) Mix well before use.

#### TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **100 µl** of standards and serums into the appropriate wells.
2. Dispense **100 µl** of Sample Diluent into each well. Incubate at 37°C for **30 minutes**.
3. After incubation, empty the microtitre wells and wash the plate 5 times with 350µl of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
4. Dispense **150 µl** of Enzyme Conjugate reagent into each well. Incubate at 37°C for **30 minutes**.
5. After incubation, empty the microtitre wells and wash the plate 5 times with 350µl of diluted wash buffer. Strike the microtitre plate sharply onto absorbent paper towel to remove all residual droplets.
6. Dispense **100 µl** of TMB substrate into each well. Incubate at room temperature(18-25°C) in the dark, for **20 minutes**.
7. Stop the reaction by adding **100 µl** of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
8. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes**.



#### CALCULATION OF RESULTS

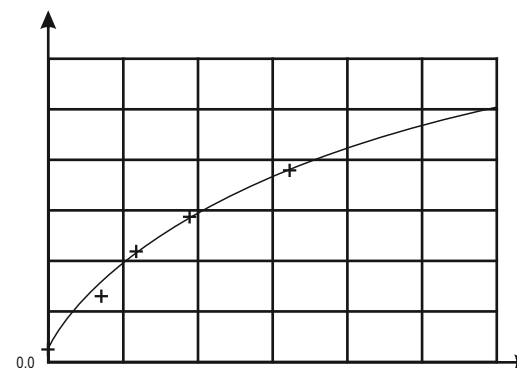
Construct a standard curve by plotting the absorbance obtained from each reference standards against its concentration in ng/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of fPSA in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

#### Example of Standard curve

Results of a typical standard run with optical density reading at 450nm (ref 600-700nm) shown in the Y axis against fPSA concentrations shown in the X axis.

**Suggest: Use 4-Parameter Standard curve to calculate sample values.**

| fPSA (ng/ml) | Absorbance |
|--------------|------------|
| A            | 0.0097     |
| B            | 0.0762     |
| C            | 0.1693     |
| D            | 0.5495     |
| E            | 1.1550     |
| F            | 1.7725     |



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 standard curve and data.

#### Expected values:

The relationship between f-PSA/t-PSA ratio and risk of prostate cancer is also age related. When Total PSA is in the range of 4.0-10.0 ng/ml the following risk interpretation is indicated:

- f-PSA/t-PSA ratio ≤ 0.10 indicates 49% - 65% risk of prostate cancer
- f-PSA/t-PSA ratio > 0.25 indicates 9% - 16% risk of prostate cancer

Multiple factors such as population, age, specificity of test method may affect interpretation of f-PSA and t-PSA values. These ranges should be used as guidelines only. Each laboratory should establish its own reference values.

#### PERFORMANCE CHARACTERISTICS

##### A) Internal Evaluation:

1. Accuracy: In an internal study **Qualisa™ fPSA** was evaluated against commercially available licensed kit with 90 random clinical samples, & **Qualisa™ fPSA** has demonstrated 100% clinical correlation with the commercially available licensed kit. Precision: **Qualisa™ fPSA** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

| Controls | No. of testings | Mean Control values with <b>Qualisa™ fPSA</b> | Coefficient of Variation (CV) |
|----------|-----------------|---|-------------------------------|
| Level 1  | 10              | 0.284   | 3.32                          |
| Level 2  | 10              | 2.76  | 7.23                          |
| Level 3  | 10              | >10   | -                             |

##### B) External Evaluation:

**Qualisa™ fPSA** ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Qualisa™ fPSA** ELISA has demonstrated 100% correlation with the reference method.

\*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd.).