Instruction for Use

【Product Name】

Free Prostate Specific Antigen (eCLIA)

【Order Information】

REF NO.	Package Size
0320501001	50T
0320501002	2×50T
0320501003	100T
0320501004	2×100T

【Intended Use】

Immunoassay for in vitro quantitative determination of free prostate specific antigen (fPSA) in human serum or plasma. The ratio of fPSA/tPSA can be used for the differential diagnosis of prostate cancer and benign prostatic hyperplasia.

Prostate Specific Antigen (PSA) is a member of human prostate kallikrein gene family, it is a serine protease that contains chymotrypsin-like activity[1, 2]. PSAwitch with a molecular weight of about 30 KD, is synthesized and secreted into semen by prostatic epithelial cells. It is one of the main components of seminal plasma[1, 3, 4]. There are three main forms of PSA in the serum: the first is free PSA (FPSA), the second is PSA binding to α-1-antichymotrypsin (ACT) called PSA-ACT, and the third is PSA binding to α-2-macroglobulin (AMG) and manifesting as immunoreactive deficiency[5, 6, 7]. Currently, only the first two can be detected by immunoassay in commercial PSA detection, so they are together defined as total PSA (tPSA)[8]. PSA is not specific to the prostate and lacks sufficient sensitivity and specificity. Although PSA has tissue specificity, its secretion will also increase in non-malignant conditions such as benign prostatic hyperplasia (BPH). Many studies have found that fPSA% is lower in prostate cancer patients than in those with other benign lesions or the normal control. The lower the relative concentration of fPSA, the higher the risk of prostate cancer in men [4, 8, 9].

fPSA/tPSA ratio can be used in the differential diagnosis of prostate cancer and benign prostatic hyperplasia and in dynamic monitoring of patients with malignant tumors to assist the judgment of disease progression or curative effect. It cannot be used as the basis for the early diagnosis or confirmed diagnosis of malignant tumors.

【Principles of the examination method】

Sandwich principle.Total duration of assay: 9 minutes.

The Free Prostate Specific Antigen (fPSA) Assay Kit adopts the double-site sandwich electrochemiluminescence method: biotinylated fPSA antibody, ruthenium (Ru) complex-labeled PSA antibody, streptavidin-coated magnetic articles and fPSA antigen in the samples are incubated together to form an antigen-antibody sandwich complex. The complex is transferred to the measuring cell and fixed on the electrode surface, and unbound substances are washed away. The electrochemical luminescence reaction is generated after the electrode is electrified, and the generated optical signal is measured by a photomultiplier and converted into an electrical signal, and then processed by an instrument, and the fPSA concentration in the sample is calculated based on the calibration curve.

【Main Components】

The reagent pack consists of MB, RA, RB, calibrators and control materials(optional), and different components and reagents of lot numbers should not be used interchangeably.

Components	Ingredients	Volume (2×50T)	Volume (50T)	Volume (100T)	Volume (2×100T)
(MB)	Streptavidin coated magnetic particles, 0.45 mg/mL; 0.1 M phosphate-buffered saline (PBS); ProClin300	2×1.8 mL	1×1.8 mL	1×3.5 mL	2×3.5 mL
(RB)	Biotinylated fPSA antibody, 0.2 mg/L; 0.1 M PBS; ProClin300	2×3.5 mL	1×3.5 mL	1×7.0 mL	2×7.0 mL
(RA)	Ru complex-labeled PSA antibody, 0.1 mg/L; 0.1 M PBS; ProClin300	2×3.5 mL	1×3.5 mL	1×7.0 mL	2×7.0 mL
Calibrator	PSA antigen, 0.1 M HEPES buffer, ProClin 300	High: 2×1.0 mL Low: 2×1.0 mL	High: 1×1.0 mL Low: 1×1.0 mL		
Control Material (Optional)	PSA antigen, 0.1 M HEPES buffer, ProClin 300	High: 2×1.0 mL Low: 2×1.0 mL	High: 1×1.0 mL Low: 1×1.0 mL		

The assignment process of supporting calibrators in this pack is strictly implemented with reference to ISO 17511:2020, which can be traced to the second-generation international standard substances for free prostate specific antigen (WHO 17/102).

Refer to the quality control card for the target value and range of the quality controls.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic).

eCL8000, eCL8000i, eCL8000p, eCL8000x:

- Auffer 480 mL
- Buffer 480 mL
- Concentrated washing buffer
- Assay cup

eCL9000, eCL9000i, eCL9600, eCL9900, eCL9900i:

- Auffer 2 L
- Buffer 2 L
- Concentrated washing buffer
- Assay cup
- Disposable sampling head
- Preconditioning bath
- Waste bags

【Storage and Shelf Life】

Unopened reagent kit, calibrators and control materials should be placed at 2~8ºC and will be valid for 18 months.

Once opened, they can be stored at 2~8ºC for 56 days, and reagent kit can also be stored in machine (4~15ºC).

The manufacturing date is labeled on the box, kit and bottles, and the expiration time is 18 months after production.

Damaged, expired or contaminated reagents should be discarded.

【Applicable Instrument】

Automated ECL Immunoassay Analyzer: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL9000, eCL9000i, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

It is recommended to use serum or plasma samples collected in blood collection tubes with heparin lithium, EDTA- 2K and EDTA- 3K anticoagulants.

Blood samples should be collected in accordance with the standard operation of venous puncture; After the sample is completely coagulated, centrifugation should be performed to remove residual cellular substances. The sample should be free of air bubbles during testing. Lipid layer covering the sample after centrifugation should be removed. It is not recommended to use hemolyzed samples.

Samples can be stored for 12 hours at room temperature (18~28 ℃), 7 days at 2~8℃, and 90 days at -15℃ or below. Samples should avoid repeated freeze-thaw cycles. Do not use the samples after two freeze-thaw cycles.

【Testing Procedure】

Testing procedure and precautions

Before testing, the system operation manual of the measuring instrument should be carefully read, so as to obtain relevant information such as system operating procedures, sample management, safety precautions and maintenance, and materials required for testing should be prepared. fPSA testing procedures should be called and set up in accordance with the system operating procedures.

Before using the reagent, put it into the analyzer 30 minutes in advance to automatically stir the magnetic bead particles and keep them in suspension.

Recommended environment temperature for testing: 10~30℃; relative humidity: ≤ 80%.

The fPSA test adopts the double-site sandwich electrochemiluminescence method, with the total test time of 9 minutes, and the test method is as follows:

Step 1: After the user applies for testing, the system automatically aspirates 70 μL of biotinylated fPSA monoclonal antibody, 70 μL of Ru complex-labeled PSA monoclonal antibody, 35 μL of streptavidin coated magnetic particles and 20 μL of sample into the reaction cup. They are automatically incubated at 37℃ for 9 min to form antigen-antibody sandwich complexes, and then the whole complexes are bound to the magnetic particles under the interaction of biotin and streptavidin.

Step 2: After incubation, the system automatically aspirates the reaction mixture into the measuring cell, the magnetic particles are captured onto the surface of electrode, while the unbound substances are washed away by buffer, and the electrodes is applied with voltage to generate chemiluminescence. The generated optical signals are measured by the photomultiplier, and the measured results are automatically determined via the calibration curve specifically generated by the instrument (this curve is obtained by performing two-point calibration for the master calibration curve obtained by reading the reagent RFID).

Calibration

Calibration should be performed using the lot-matching reagent and calibrators.

The target value of the calibrator has been written into the Radio-Frequency Identification card (RFID card) of the kit.

1. Before calibration, the target value, reagent information and master calibration curve information of the calibrator in the RFID card (Radio Frequency Identification, inductive electronic chip or proximity card) of the kit should be imported into the test system by swiping the card.
2. The supporting calibrator is tested, and the analyzer adjusts the master calibration curve according to the test results of the supporting calibrator to obtain the calibration curve tested by the current system (the analyzer can automatically interpret the validity of the calibration curve according to the adjustment results).

The test system should re-perform the calibration operation under the following conditions:

(1) When different lots of reagents are used;

(2) When the same lot of reagents has been used on the analyzer for more than 28 days;

(3) According to requirements: e.g. if the quality control result exceeds the defined limit.

(4) When changing the buffers of different lot numbers.

Quality Control procedure

In order to ensure the reliability of test results, it is recommended to test the control materials every 24 hours. It is recommended to test the control materials after each calibration, reagent lot replacement, maintenance or troubleshooting. The quality control results should all fall within the defined range. If they exceed the defined range, the reasons such as instrument status, reagents and calibrators should be investigated and the corrective measures should be taken.

Calculation

The system software can automatically calculate the analyte concentration, with the result in ng/mL.

Specimen dilution

The detection range is relatively wide, and the sample does not need to be diluted.

【Biological Reference Interval】

By analyzing the serum samples of 313 healthy males from hospitals, it was

calculated that the upper limit of the reference range of 95% is 1.0 ng/mL.
Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

【Interpretation of results】

In prostate cancer screening, 123 men with average age ≥ 50 years simultaneously underwent fPSA and tPSA detection, and it was found that when the tPSA concentration was (4-10) ng/mL, the fPSA/tPSA was < 0.1. ROC analysis proved that 95% was clinically specific.
When interpreting the test results, it is necessary to refer to the patient's overall clinical situation, including: symptoms, medical history as well as other corresponding data and information.

【Limitations】

The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.
The detection range of the kit is 0.01 ng/mL~50 ng/mL. If the fPSA concentration in the sample is lower than the lower limit of detection, the result is reported as < 0.01 ng/mL; if the fPSA concentration in the sample is higher than the upper limit of detection, the result is reported as > 50 ng/mL.
When jaundice (bilirubin) is ≤ 65 mg/dL, hemolysis (hemoglobin) is ≤ 500 mg/dL, lipemia (triglyceride) is ≤ 1500 mg/dL, biotin is ≤ 20 ng/mL, and HAMA is ≤100 ng/mL in the sample, the interference deviation of the test results is within ±15%.
When the concentration of rheumatoid factor in the sample is ≤ 1500 IU/mL, the relative recovery of the test results is between 85% and 115%.
When the sample contains leuprorelin acetate (100 μg/mL), cyclophosphamide (700 μg/mL), finasteride (370 ng/mL), megestrol acetate (2.4 mg/dL), methotrexate (30 μg/ mL), flutamide (10 μg/mL), doxorubicin hydrochloride (16 μg/mL), aspirin (0.5 mg/mL), and diethylstilbestrol (2 μg/mL), the interference deviation of the measured results is within ±10%.
When the fPSA antigen concentration reaches 15000 ng/mL, the test results are not affected by the hook effect.

【Analytical Performance】

Lower limit of measurement

Limit of Blank (LoB) ≤ 0.01 ng/mL.

Accuracy

The recovery sample added with an appropriate concentration is tested, and the recovery should be within the range of 85%~115%;
the national standard of fPSA is tested, with the ratio of measured value to theoretical value within the range of 0.85~1.15.

Linearity

Within the range of 0.01 ng/mL ~ 50 ng/mL, the correlation coefficient (r) of the kit should not be less than 0.9900.

Within-run imprecision (repeatability)

The coefficient of variation (CV) is less than 5%.

Between-lot imprecision

The coefficient of variation (CV) is less than 10%.

Analytical specificity

Use the national standard of fPSA and the national standard of PSA to prepare the samples of serial concentrations, and the deviation between the test concentration and the theoretical concentration is within ±15%.

Homogeneity of calibrators and control materials

In-bottle homogeneity: The standard deviation (SD) of low-point calibrator is ≤ 1.0 ng/mL, and the coefficient of variation (CV) of high-point calibrator, high-value quality control and low-value quality control is ≤ 5%.
Between-bottle homogeneity: The standard deviation (SD) of low-point calibrator is ≤ 1.0 ng/mL, and the coefficient of variation (CV) of high-point calibrator, high-value quality control and low-value quality control is ≤ 8%.

Accuracy of calibrators

The supporting calibrators of the kit are tested, the relative deviation of their test results is within ±15%.

Measured values of quality control

The measured results of supporting assigned quality control of the kit should be within the quality control range.

【Precaution and Warning】

This kit is only used for in vitro diagnosis;
When using this kit, the relevant operation precautions of the laboratory must be observed;
The test results of this kit can only be used as clinical reference, the patient's clinical evaluation should be based on the patient's clinical symptoms/signs, medical history, other laboratory test results and treatment response, etc.;
Due to methodological reasons or antibody specificity, the results of the same sample tested with reagents of different manufacturers may be different. Therefore, results obtained with different kits should not be directly compared, so as to prevent wrong medical interpretation; it is recommended that the Laboratory Department indicate the characteristics of the reagents in the test report issued to the clinician. If the reagent type is changed during series monitoring, continuous testing should be conducted, and the results should be compared with the original reagent results in parallel to re-determine the baseline value;
This product contains animal-derived substances, and may have potential biological risks. All samples and reaction wastes should be treated as the source of infection, and all wastes must be disposed of in accordance with local regulations.

【Symbol】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Authorized representative in the European Community		In vitro diagnostic medical device
	Use-by date		Indicates this device is in compliance with Europe Directive
	Batch code		Contains sufficient for<*n*>tests
	Serial number		Biological risks
	Temperature limitation		This way up
	Catalogue number		

【Bibliography】

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【Manufacturer】

Shenzhen Lifotronic Technology Co., Ltd.
Unit A, 4th Floor, Building 15, Yijing Estate, No.1008 Songbai Road, Nanshan District, Shenzhen City, Guangdong Province, 518055, P.R.China
E-mail: inter-service@lifotronic.com

【European Representative】

Shanghai International Holding Corp. GmbH (Europe)
Eiffestrasse 80, 20537 Hamburg, Germany
Tel: +49-40-2513175 Fax:+49-40-255726

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