EPITOPE DIAGNOSTICS, INC.

EDI™ Free Prostate-specific antigen CLIA kit

Chemiluminescence Immunoassay (CLIA) for the quantitative measurement of free PSA in Serum.

REF CL0816R RUO 50, 100, 250

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of human Free Prostate-specific antigen levels in serum using the ECL100 or ECL25 Immunoassay analyzer. This immunoassay is indicated for measurement of free PSA in conjunction with the CL0815R EDI™ Total Prostate-specific antigen CLIA kit to develop a ratio of Free PSA to Total PSA. This ratio is used as an aid in the detection of prostate cancer in men.

For Research Use only

SUMMARY OF PHYSIOLOGY

Several forms of prostate-specific antigen (PSA) are found in serum. Free PSA is one of these, and unlike other forms, is unbound to protease inhibitors. The measurement of the different complexed and uncomplexed forms helps distinguish between prostate cancer and other non-cancerous diseases[1]. The determination of PSA levels present is serum can be used for screening and determination if a more invasive biopsy is required, and additionally, can be used for monitoring patients with prostate cancer[2].

ASSAY PRINCIPLE

This CLIA is designed, developed, and produced for the quantitative measurement of human free PSA in serum samples. The assay utilizes a two-site "sandwich" technique with one antigen and one antibody that bind to different epitope and paratope of free PSA.

Assay calibrators, controls, or patient samples are added directly to a reaction vessel containing streptavidin coated magnetic particles. Simultaneously, an acridinium ester antibody and a biotin antibody are added. The magnetic particles capture the biotin antibody as well as an immunocomplex in the form of "magnetic particles – biotin free PSA antibody –free PSA– acridinium ester free PSA antibody".

The materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the trigger solution is added to the reaction vessel and light generated by the reaction is measured with the ECL100 or ECL25 analyzer. The relative light units (RLU) are proportional to the concentration of free PSA in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in serum free PSA concentration.

REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date. Reagents from different kit lot numbers should not be combined or interchanged.

Standard Batch Quantity: 100/kit

1. Free PSA Magnetic Particle Solution (L0647)

Qty: 1 x 1.55 mL (50/kit), 1 x 2.8 mL (100/kit), 1 x 6.55 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

2. Biotin Free PSA Antibody (L0648)

Qty: 1 x 4.75 mL (50/kit),1 x 8.5 mL (100/kit), 1 x 19.75 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

3. Acridinium Ester Free PSA Antibody (L0649)

Qty: 1 x 4.75 mL (50/kit), 1 x 8.5 mL (100/kit), 1 x 19.75 mL (250/kit)
Storage: 2 – 8°C
Preparation: Ready to Use

4. Free PSA Calibrators (L0650 – L0651)

Liquid human free PSA in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty: 2 x vials of 0.5 mL each
Storage: 2 – 8°C
Preparation: 0.5 mL of Calibrators, mix by inversions or gentle vortexing. Make sure that Calibrators are well mixed before use.

5. Free PSA Controls (L0652 – L0653)

Liquid human free PSA in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty: 2 x vials of 0.5 mL each
Storage: 2 – 8°C
Preparation: 0.5 mL of Controls, mix by inversions or gentle vortexing. Make sure that Controls are well mixed before use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

The instrument only uses materials supplied by Epitope Diagnostics, Inc. When materials available from third-party suppliers are used, Epitope Diagnostics, Inc. takes no responsibility for the validity of results obtained. Material is

available for purchase from Epitope Diagnostics, Inc. Please contact your distributor for more information.

1. ECL100 Immunoassay Analyzer or ECL25 Immunoassay Analyzer
2. CL011 Cuvettes (for ECL100) or CL010 Cuvettes (for ECL25)
3. Wash Reagent (P-594)
4. Trigger Solutions A and B (P-595)

SPECIMEN COLLECTION AND PREPARATION

Only 10 µL of human serum or plasma sample is required for free PSA measurement in singlet. Samples should not be taken from patients taking biotin-containing multivitamins or dietary supplements at least 48 hours prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500 RPM for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 15-25°C for three days, 2-8°C for five days, and –20°C or below for three months. Avoid more than three freeze-thaw cycles of specimen. It is necessary to take care in the sample collection procedure to avoid hemolysis.

Some substances in the samples will interfere with the test results. The common interfering substances and maximum allowable concentrations are as follows:

- bilirubin 60 mg/dL
- triglycerides 1500 mg/dL
- hemoglobin 900 mg/dL
- biotin 200 nmol/L
- For patients receiving high-dose biotin therapy (5 mg/ day), samples must be collected 8 hours after taking the last dose of biotin

A single assay of this item requires 10 µL sample. This quantity does not include the dead volume in the sample container, the capacity required for retesting, and other measurement items. For the definition of the minimum required sample size, refer to the equipment manual.

CALIBRATION

An active calibration curve is required for all tests. For the assay, calibration is required for the first-time use of a reagent lot and is valid for 28 days. However, we recommend calibration every 14 days after initial calibration or when either kit control is out of range.

QUALITY CONTROL

The characteristics of patient samples are simulated through controls and are critical to validate the performance of CLIA assays due to the random-access format. Use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. We suggest performing a control test once every day. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator and control vials.

2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges.

2.2 For the ECL100, take out the Magnetic Particle bottle and make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance.

- Note: For ECL 100, if the Magnetic Particle Solution volume is over 3 mL, it will be provided in a glass bottle. It will need to be transferred from the glass bottle to the plastic vial in the cartridge with the rest of the reagents. Make sure the Magnetic Particle Solution is mixed well before transferring.

2.3 For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber.

2.4 The reagents are now ready to be loaded into the ECL100 or ECL 25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 or ECL25 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
Free PSA Calibrators (L0650-L0651)	10	-
Samples	-	10
Free PSA Magnetic Particle Solution (L0647)	25	25
Biotin Free PSA Antibody (L0648)	75	75
Incubation Period 1		
Wash the reaction cup 3 times with the wash reagent.		
Acridinium Ester Free PSA Antibody (L0649)	75	75
Incubation Period 2		
Wash the reaction cuvette 3 times with wash reagent.		
Trigger Solution A (P-595)	200	200
Trigger Solution B (P-595)	200	200

The total incubation time is less than 30 minutes.

INTERPRETATION OF RESULTS

The chemiluminescence analyzer calculates the concentration values of the sample and the control by a standard curve (fitting method: four parameters or point-to-point) and the measured RLU. Values are compared with the range of the marked value. If it exceeds the indicated quality control range, it indicates that the test is unqualified and needs to be re-tested.

Due to methodological differences or antibody/antigen specificity, there may be deviations between the test results of reagents from different manufacturers. Therefore, direct comparisons should not be made to avoid false interpretation.

EXPECTED VALUES

Free PSA concentrations were measured in serum samples collected from 125 apparently healthy adults using the EDI™ Human Free Prostate-specific antigen CLIA Kit. The observed range of free PSA when the total PSA concentration is 10.0 ng/mL is summarized in the table below.

Age	Free PSA Cutoff Concentration (ng/mL)	Free/Total PSA Ratio
50-59	1.0	0.1
60-69	1.5	0.15

70+	1.0	0.1

It is highly recommended that each laboratory should establish their own normal range for free PSA based on local populations.

LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL25 Immunoanalyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different lots cannot be mixed.
3. Test results from this product should not be the sole basis for clinical diagnosis.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measurement result is recalculated according to the dilution ratio to ensure the accuracy of the result.
5. When the sample concentration of free PSA is lower than the detection lower limit, the test result can be reported as <0.09 ng/mL. When the sample concentration is higher than the detection upper limit, it can be reported as >176.49 ng/mL.

PERFORMANCE CHARACTERISTICS

Hook Effect

The assay shows no hook effect up to 30,003.30 ng/mL.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates in three assays of calibrator matrix to be 0.04 ng/mL.

Limit of Detection

The limit of detection (LoD) was determined by 60 replicates in three assays of low-level samples to be 0.09 ng/mL.

Limit of Quantification

The limit of quantification (LoQ) was determined by 60 replicates in three assays of low-level samples to be 0.15 ng/mL.

Linearity

Linearity was determined by two assays with a diluted standard of high free PSA concentration. In each assay, the average of two replicates of each of the diluted samples is used for a correlation analysis against calculated theoretical values. The linearity of this test is up to 176.49 ng/mL.

Standard	Average Concentration (ng/mL)	Theoretical Concentration (ng/mL)	Linear Recovery (%)	R^2
1	0.00	0.00	100	1.000
2	1.97	1.84	107	
3	3.78	3.68	103	
4	7.12	7.36	97	
5	14.08	14.71	96	
6	42.61	44.12	97	
7	90.60	88.25	103	
8	179.00	176.49	101	

Intra-assay Precision

Precision was determined by measuring eight replicates of three specimens. The results are as follows:

Sample	Average Concentration (ng/mL)	SD	CV (%)
1	3.74	0.15	4.1
2	29.54	0.56	1.9
3	187.90	7.51	4.0

Inter-assay Reproducibility

Reproducibility was determined by measuring three specimens in twenty-four replicates over the run of three assays. The results are summarized below:

Sample	Average Concentration (ng/mL)	SD	CV (%)
1	3.74	0.17	4.5
2	28.56	1.09	3.8
3	172.27	15.52	9.0

Cross Reactivity

Cross-reactivity was assessed by analyzing several specimens containing several analytes at elevated concentrations. The results are summarized below:

Analytes	Theoretical Concentration	Measure Concentration (ng/mL)
Human Epididymal protein 4	1505.52 pmol/L	0.16
Human Alphafetoprotein	964.45 IU/mL	0.13
Human Squamous cell Carcinoma Antigen	68.08 ng/mL	0.10
Cancer Antigen 72-4	318.27 U/mL	0.13

Interference

Bilirubin, hemoglobin, and lipid triglycerides were tested as potential interferents to free PSA. Randomly selected samples were spiked with the potential interferents at the concentrations listed in the table below:

Interferent (Concentration tested, mg/mL)		Test (ng/mL)	Control (ng/mL)	Bias (d_{obs}, %)
Bilirubin	0.005	3.06	3.01	1.7
		26.37	26.20	0.7
	0.01	3.00	3.01	-0.2
		26.14	26.20	-0.2
	0.02	2.97	3.01	-1.2
		26.53	26.20	1.3
Hemoglobin	0.5	3.33	3.35	-0.7
		28.67	28.93	-0.9
	1.0	3.19	3.35	-4.8
		27.90	28.93	-3.5
	2.0	3.23	3.35	-3.7
		28.50	28.93	-1.5
Lipids	1.0	3.28	3.45	-4.9
		29.53	29.87	-1.2
	5.0	3.20	3.45	-7.1
		31.25	29.87	4.6
	10.0	3.39	3.45	-1.7
		26.33	29.87	-11.9

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

1. Catalona WJ, Partin AW, Slawin KM, et al. Use of the Percentage of Free Prostate-Specific Antigen to Enhance Differentiation of Prostate Cancer From Benign Prostatic Disease: A Prospective Multicenter Clinical Trial. *JAMA.* 1998;279(19):1542–1547. doi:10.1001/jama.279.19.1542
2. James G. Huang, MD, PGDipSurgAnat, FRACS,, Nicholas Campbell, MD, FRACS,, S. Larry Goldenberg, MD, FRCSC, FACS, FCAHS. PSA and beyond: Biomarkers in prostate cancer. BCMJ, Vol. 56, No. 7, September, 2014, Page(s) 334-341 - Clinical Articles.

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

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to learn more about our products and services.

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

RUO For Research Use Only	CE European Conformity	**LOT** Lot Number
REF Catalog Number	Read instructions before use	Number of Tests
Store at	Use by	Keep away from heat and direct sun light
Manufacturer	**EC REP** Authorized Representative in Europe	