CTK

tPSA ELISA

IVD | REF E2003

Instructions for Use

- 96-well ELISA kit for the quantitative determination of Total Prostate Specific Antigen (tPSA) concentration in human serum
- For export only, not for re-sale in the USA
- Store at 2-8°C upon receipt

INTENDED USE

The *CTK* tPSA ELISA is a solid-phase enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of total prostate specific antigen (tPSA) concentration in human serum. It is intended to be used by professionals as an aid in the diagnosis of prostate dysfunction.

INTRODUCTION

Prostate Specific Antigen (PSA) is a single chain glycoprotein, named for its regular appearance as an antigen of the prostate[1,2,3], and lack of appearance in any other bodily tissue.

Determination of PSA concentration in serum is critical as it is useful in finding the presence of prostate cancer and can also assess the response to on-going treatment. PSA is found in all forms of prostate cancer, the most commonly diagnosed cancer in men. Prostate cancer is also the second leading cause of cancer-based deaths in the male population. Diagnosis of altered levels of PSA can aid in early detection of prostate cancer and other disorders[4,5,6]. PSA tests are considered more effective than prostatic acid phosphatase due to the increased sensitivity of the PSA ELISA[4,7].

CTK tPSA kit contains calibrators with known concentrations of tPSA and PSA specific antibodies conjugated to Biotin and Horseradish peroxidase (HRP). These components enable a dose response curve based on the concentration of the calibrators and OD values. Patient samples concentration is determined by interpolating unknown specimens OD value on this curve.

TEST PRINCIPLE

The *CTK* Total Prostate Specific Antigen (tPSA) ELISA is a sandwich solid-phase enzyme-linked immunosorbent assay for the quantitative measurement of tPSA concentration in human serum.

The *CTK* Total Prostate Specific Antigen (tPSA) ELISA is comprised of three key components:

1) Solid microwells pre-coated with streptavidin
2) tPSA Calibrators
3) Enzyme conjugate containing anti-PSA labelled with Biotin (Bio-Ab) and Anti-PSA labelled with HRP (HRP-Ab)

During the assay, the test specimen is added to the SA coated microwell along with the enzyme reagent. The PSA in the patient specimen binds to both Bio-Ab and HRP-Ab, forming a sandwich immunocomplex. The biotin in the Bio-Ab conjugate binds to the SA on the microwell surface, anchoring the immunocomplex.

Any unbound material is then removed by washing. TMB Substrate is added and the presence of the HRP bound to the microwell surface is shown by the development of blue color. The reaction is then terminated with the Stop Solution and the optical density (OD) is determined using a spectrophotometer at 450/610-650 nm.

A standard curve can then be developed by plotting the tPSA Calibrator concentrations on the x-axis against the OD values on the y-axis. The tPSA concentration of or test specimen can be interpolated from the standard curve.

MATERIALS AND REAGENTS

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 25 µL, 50 µL,100 µL, and 1 mL
2. Microplate reader
3. Vortex mixer or equivalent
4. Absorbent paper for blotting the microwells
5. Graph paper
6. Timer
7. Distilled or de-ionized water

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Streptavidin Coated Microwells	8 wells x 12 strips	E2003W
2	tPSA Calibrators: C1 (0 ng/mL)	1 mL	E2003C1
3	C2 (5.0 ng/mL)	1 mL	E2003C2
4	C3 (10.0 ng/mL)	1 mL	E2003C3
5	C4 (25.0 ng/mL)	1 mL	E2003C4
6	C5 (50.0 ng/mL)	1 mL	E2003C5
7	C6 (100.0 ng/mL)	1 mL	E2003C6
8	tPSA Assay Control*	1 mL	E2003AC
9	tPSA Enzyme Reagent	12 mL	E2003H
10	Wash Buffer Concentrate (40X)	25 mL	WE4001-25
11	TMB Substrate	12 mL	TME2004
12	Stop Solution	7 mL	SE1002-7
13	Instructions for Use	1	PI-E2003
14	ELISA Working Sheet	2	E0001ES
Others	2 x Microplate Sealers and 1 x Resealable Plastic Bag		

**The Assay control in this test is lot specific. Please see vial labels for exact concentrations.*

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. Ensure that the reagents are brought to room temperature (20-28°C) before opening. After removing the desired number of wells, place and seal unused wells in the resealable plastic bag provided with desiccant and return to 2-8°C. All reagents are stable through the expiration date printed on the label if not opened. Once opened, the kit is stable for 8 weeks at 2-8°C, or until the labeled expiration date, whichever is earlier.

SPECIMEN COLLECTION AND PREPARATION

- Serum specimens should be prepared from whole blood obtained by acceptable venipuncture technique.
- If not tested immediately, the specimens can be stored at 2-8°C for up to 7 days. The specimens should be frozen at ≤ -20°C for longer storage. Avoid multiple freeze-thaw cycles. If a specimen is to be shipped, pack in compliance with federal regulations covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation before testing.
- Do not use specimens demonstrating gross lipemia, gross hemolysis or turbidity.
- Specimens containing sodium azide may interfere with test results.

PREPARATION OF THE REAGENTS

1. Bring all reagents to room temperature (20-28°C).
2. **Preparation of working Wash Buffer:**
   If precipitants are visible, warm up the Wash Buffer (40X concentrate) at 37°C. Dilute concentrated Wash Buffer 40-fold with water as follows:

Plate	DI water	40X wash buffer	Final volume
Full plate	195 mL	5 mL	200 mL
Half plate	97.5 mL	2.5 mL	100 mL
Quarter plate	48.75 mL	1.25 mL	50 mL

3. Mix each reagent before adding to the test wells.
4. Determine the number of strips needed and mark on the ELISA working sheet with the appropriate information. **Calibrators should be run in duplicate to ensure accuracy.**

ASSAY PROCEDURE

1. Calculate the desired number of microwells. Remove the remaining microwells and place them with desiccant into the resealable plastic bag, seal and store at 2-8°C for later use.
2. Add 25 µL of tPSA calibrators, tPSA Assay Control, and test specimen(s) into the designated wells.
3. Add 100 µL of tPSA Enzyme Reagent into all wells.
4. Cover the plate with a microplate sealer, and then shake gently for 10 seconds.
5. Incubate the microplate at room temperature (20-28°C) for 30 minutes.
6. Wash Step (Can be performed manually or with automated washing):
   Manual washing: Carefully remove the incubation mixture by disposing the solution into an appropriate waste container. Fill each well with 350 µL of working wash buffer and mix gently for 20-30 seconds. Discard the wash solution completely. Repeat 2 more times. After completing the last wash step, tap the plate face-side down on absorbent paper to remove residual liquid.

   Automated washing: Automatic plate washer must be calibrated to ensure efficient washing. Fill each well with 350 µL of diluted wash buffer. Aspirate all wells completely. Repeat 2 more times.
7. Add 100 µL of TMB Substrate into each well.
8. Cover the microplate and incubate at room temperature (20-28°C) for 15 minutes.
9. Stop the reaction by adding 50 µL of Stop Solution to each well. Gently mix for 10 seconds. Add the Stop Solution in the same sequence as substrate addition. **It is important to make sure that all the blue color changes completely to yellow color.**
10. Set the microplate reader wavelength at 450 nm. Measure the OD value of each well within 30 minutes after adding Stop Solution. A filter of 610-650 nm can be used as a reference wavelength to optimize the assay result.

Flow chart of assay procedure

1.	Secure strips in microwell frame	Number of strips
2.	Add tPSA Calibrators, tPSA Assay Controls, or specimens	25 µL
3.	Add Enzyme Reagent	100 µL
4. 5.	Gently Shake Incubate	10 seconds 20-28°C, 30 minutes
6.	Wash: manual or automatic	3 times (350 µL)
7.	Add TMB Substrate	100 µL
8.	Incubate in dark	20-28°C, 15 minutes
9.	Add Stop Solution. Gently mix	50 µL 10 seconds
10.	Read the result	450/610-650 nm within 30 minutes

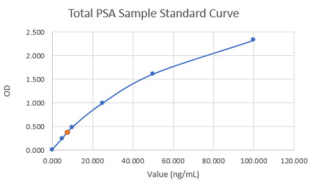
CALCULATION OF RESULTS

1. Calculate the mean OD value (A450/610-650) for each set of calibrators.
2. Construct a standard curve by plotting the mean OD value obtained for each calibrator against its concentration on graph paper with OD values on the vertical *y*-axis, and concentrations on the horizontal *x*-axis.
3. Interpolate concentrations for the assay controls and test specimens by plotting their mean OD values on the curve.
   Alternatively, if software is used, calculate the concentration of tPSA following the software menu.
4. If the OD value of a specimen is greater than that of the highest calibrator, it is recommended to dilute the specimen with C1 in a 1:1 or 1:3 ratio and test again. Any values obtained for a diluted sample must be further converted by applying the appropriate dilution factor in the calculation.

INTERPRETATION

1. Results of a typical standard curve are shown below:

Cal ID	Conc. (ng/mL)	OD
C1	0.0	0.004
C2	5.0	0.243
C3	10.0	0.482
C4	25.0	0.992
C5	50.0	1.608
C6	100.0	2.327
Patient ID	**Conc. (ng/mL)**	**OD**
1	8.76	0.412



The above data and figure are for example purposes and should not be used to calculate your result.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to check assay performance. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

The following criteria should be met to consider assay results to be valid:

- The mean OD of the '0' calibrator should be ≥ 0.100.
- CV's for duplicates of calibrators should be ≤ 10%.
- tPSA Assay Control concentrations should be within the specified range on the vial labels.

NORMAL REFERENCE

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local patient population and its own assay technique. The following values for the tPSA ELISA can be used as initial guideline ranges only:

Normal values for tPSA (ng/mL): <4.0 ng/ml

PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

Twenty (20) replicates of the '0' calibrator were run in a single assay on each of three lots of the *CTK* tPSA ELISA, and the mean and standard deviation (SD) of the OD values were calculated. The analytical sensitivity of the test was interpolated from the dose response curve. The analytical sensitivity of the *CTK* tPSA was determined to be .06 ng/mL at 2SD.

2. Specificity

Specificity of the tPSA ELISA was evaluated by spiking potential-interfering substances into a human serum matrix. Specificity was calculated by deriving a ratio between the concentration of each tested metabolite and the concentration of tPSA needed to displace the same amount of conjugate. There was no cross-reactivity observed at the concentrations listed below:

Acetylsalicylic Acid	100 µg/mL
Ascorbic Acid	100 µg/mL
Biotin	20 ng/mL
Caffeine	100 µg/mL
Carcinoembryonic antigen	10 µg/mL
Alpha-fetoprotein	10 µg/mL
Cancer antigen 125	10000 U/mL

3. Accuracy

The accuracy of the tPSA ELISA was determined by external comparison of 65 specimens with varying concentrations of tPSA with a reference commercial ELISA kit. The results are listed in the following table:

Method	Mean (ng/mL)	Least Square Regression	Corr. Coef. (r)
***CTK* (y)**	1.80	Y=0.9095x-0.038	r=0.997
Reference (x)	2.02		

4. Precision

a. **Intra-assay precision:** Twenty replicates of each of three pooled human serum controls (low, medium, and high concentrations) were tested and the mean, SD, and coefficient of variation (CV%) were determined. The results are shown in the following table:

Sample	N	Mean (ng/mL)	SD	CV%
Low	20	1.05	0.03	3.56
Medium	20	1.61	0.06	4.06
High	20	3.12	0.12	3.86

b. **Inter-Assay Precision:** Three human serum pooled controls (low, middle, and high concentrations) were assayed in separate runs over 3 lots. The mean, SD, and CV% were determined. The results are shown in the following table:

Sample	N	Mean (ng/mL)	SD	CV%
Low	16	0.75	0.055	7.3
Medium	16	3.26	0.316	9.7
High	16	19.79	0.675	3.4

5. Hook Effect:

No hook effect was observed up to 3200 ng/mL

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to inaccurate test results.
2. Do not use expired test kits.
3. Bring all reagents to room temperature (20-28°C) before use.
4. Do not use a component from any other test kit as a substitute for the components in this kit. Do not re-use the used microwells to test new samples.
5. Do not use serum derived from hemolyzed blood specimens for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mouth. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. All specimens and materials used to perform this test must be disposed of as bio-hazardous waste.
10. Prior to the first incubation, and after addition of the Stop Solution, gently tap the microwells to ensure thorough mixing. Avoid splashing liquid while shaking. Do not allow formation of air bubbles in the microwell.
11. Do not allow the microwells to dry between the end of the washing operation and the reagent distribution.
12. The enzyme substrate reaction is very sensitive to metal ions. Thus, do not allow any metal elements to come into contact with the conjugate or TMB Substrate.
13. The enzyme-substrate reaction is temperature dependent. Ensure that the room temperature is between 22-28˚C during the TMB incubation.
14. The TMB substrate must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate must be stored in the dark.
15. Use a new dispensing tip for each specimen. Never use the specimen container to distribute conjugate and TMB Substrate.
16. **The wash procedure is critical. Wells must be aspirated completely before adding the Wash buffer or liquid reagents. Automatic washers must be validated with the test kit prior to use. Insufficient washing will result in poor precision and falsely elevated OD values.**
17. **Microplate reader must be calibrated per manufacturer's instruction to ensure accurate OD readings. A non-calibrated reader may lead to invalid test results.**
18. Avoid exposure to strong light during color development.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Results must be followed closely when testing for tPSA concentration in human specimens. Failure to follow the procedure may lead to inaccurate results.
2. The test is limited to the detection of tPSA concentration in human serum.
3. A clinical diagnosis should not be based on the results of a single test and should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. Normal PSA levels do not exclude disease.
5. If the OD value of a specimen is greater than that of the highest calibrator, it is recommended to dilute the specimen with Calibrator C1 and test again.
6. Any interpretation or use of this test result must also integrate other clinical findings as well as on the professional judgment of health care providers.
7. Patients should be instructed to avoid taking biotin at least 72 hours prior to test as it might interfere with the results.

REFERENCES

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4. Wild D, *The Immunology Handbook*, Stockton Press, 452 (1994)
5. Junker R, Bradt B,Zechel C, Assmann G, *Comparison of Prostate-Specific antigen (PSA) measured by four combinations of free PSA and total PSA assays,* Clinical Chemistry, 43 1588-94 (1997)
6. Wians FH. *Clinical Laboratory Tests: Which, Why, and What Do The Results Mean?* Lab Med 2009; 40(2):105-113.
7. Ilic D, et. Al, *Prostate Cancer Screening with prostate-specific antigen (PSA) test: a systematic review and meta-analysis*, BMJ, 362, k3519 (2018)

Index of Symbols

See instructions for use		Use by	
IVD	For *in vitro* diagnostic use only	CAL	Calibrator
REF	Catalog #	CONJ	Conjugate
LOT	Lot number	IA CONTROL	Assay control
Σ N	Tests per kit	TMB SUBS	TMB substrate
	Do not reuse	STOP SOLN	Stop solution
	Manufacturer	WASH BUFF	Wash buffer
	Date of manufacture	MICROWELLS	Coated microwells
2°C 8°C	Store between 2-8°C		

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