



EAGLE
BIOSCIENCES

Total Prostate Specific Antigen (tPSA) LIA Assay Kit

Catalog Number:

PSA31-L01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 1.0

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INTENDED USE

The Eagle Biosciences Total Prostate Specific Antigen (tPSA) LIA Assay Kit is for the direct quantitative determination of tPSA in human serum by a chemiluminescence immunoassay (LIA). The Eagle Biosciences Total Prostate Specific Antigen (tPSA) LIA assay kit is for research use only and not to be used in diagnostic procedures.

PRINCIPLE OF THE ASSAY

The principle of this chemiluminescence immunoassay (LIA) test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for total PSA is immobilized onto the microplate and another monoclonal antibody specific for a different region of PSA is conjugated to horse radish peroxidase (HRP). Total PSA from the sample and standards are allowed to bind to the plate, washed, and subsequently incubated with the HRP conjugate. After a second washing step, the luminescence substrate is added and the relative luminescence units (RLUs) are measured in a microplate luminometer. The RLUs formed by the enzymatic reaction are directly proportional to the concentration of total PSA in the samples. A set of standards is used to plot a standard curve from which the concentrations of tPSA in patient and control samples are read.

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The kit controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges. The performance of this assay is markedly influenced by the correct execution of the washing procedure!
9. The luminescence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
10. When dispensing the substrate, do not use pipettes in which these liquids will come into contact with any metal parts.
11. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
12. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
13. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.



LIMITATIONS

1. All the reagents within the kit are calibrated for the determination of tPSA in human serum. The kit is not calibrated for the determination of tPSA in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. This kit is intended for research use only and should not be used in diagnostic procedures.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be nonreactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid direct contact with reagents. In case of contact, wash with plenty of water.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.2 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 25, 100 and 300 μ L
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate luminometer

REAGENTS PROVIDED AND PREPARATION

1. Mouse Anti-PSA Monoclonal Antibody-Coated Break-Apart Well Microplate — Ready To Use

Contents: One 96-well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.



2. Mouse Anti-PSA Monoclonal Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation X100

Contents: Anti-PSA monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 0.3 mL/vial

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

Preparation of conjugate working solution: Dilute conjugate concentrate 1:100 in assay buffer before use (example: 20 µL of conjugate concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 120 µL of conjugate concentrate in 12 mL of assay buffer. Discard any that is left over.

3. PSA Calibrators — Ready To Use

Contents: Eight vials containing PSA in a protein-base buffer with a non-mercury preservative. Prepared by spiking buffer with an exact quantity of PSA. Calibrated against World Health Organization (WHO) 1st IS 96/670 (90:10).

* Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 ng/mL	2.0 mL
Calibrator B	0.1 ng/mL	0.5 mL
Calibrator C	0.4 ng/mL	0.5 mL
Calibrator D	1.6 ng/mL	0.5 mL
Calibrator E	4 ng/mL	0.5 mL
Calibrator F	16 ng/mL	0.5 mL
Calibrator G	40 ng/mL	0.5 mL
Calibrator H	100 ng/mL	0.5 mL

Storage: Refrigerate at 2–8°C.

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. Controls — Ready To Use

Contents: Two vials containing PSA in a protein-base buffer with a non-mercury preservative. Prepared by spiking buffer with an exact quantity of PSA. Refer to vial labels for acceptable range.

Volume: 0.5 mL/vial

Storage: Refrigerate at 2–8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate – Requires Preparation X10

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.



Preparation: Dilute 1:10 in distilled or deionized water before use. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. Assay Buffer

Contents: One bottle containing protein-based buffer with a non-mercury preservative.
Volume: 30 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.

7. LIA Substrate Reagent A – Requires Preparation

Contents: One vial containing luminol plus enhancer.
Volume: 0.8 mL/vial
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: See preparation of LIA working substrate solution.

8. LIA Substrate Reagent B – Requires Preparation

Contents: One vial containing stabilized peroxide solution.
Volume: 1.6 mL/vial
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: See preparation of LIA working substrate solution.

9. LIA Substrate Reagent C – Requires Preparation

Contents: One bottle containing buffer with a non-mercury preservative.
Volume: 16 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: See preparation of LIA working substrate solution.

PREPARATION OF LIA WORKING SUBSTRATE SOLUTION

In a clean plastic container (glass is not suitable) mix 1 part of LIA substrate reagent A with 2 parts of LIA substrate reagent B and 20 parts of LIA substrate reagent C. This gives the ready to use substrate solution. If the whole plate is to be used prepare working substrate solution as follows:

Combine 0.75 mL of LIA substrate reagent A with 1.5 mL of LIA substrate reagent B and 15 mL of LIA substrate reagent C. It is suggested to wait at least 30 minutes prior to use after preparation of the working substrate solution. The working substrate solution is stable for up to 8 hours at room temperature. Discard the leftovers.



ASSAY PROCEDURE

Important Notes:

- All reagents must reach room temperature before use.
 - Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.
 - The washing procedure influences the precision markedly; it is essential to ensure the washing is effective and thorough.
1. Prepare working solutions of the conjugate, wash buffer and LIA substrate (refer to reagents provided and preparation section).
 2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
 3. Pipette 50 μ L of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
 4. Pipette 100 μ L of the assay buffer into each well. (We recommend using a multichannel pipette.)
 5. Cover the plate and incubate for 60 minutes on a plate shaker (approximately 200 rpm) at room temperature.
 6. Wash the wells 5 times with 300 μ L of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
 7. Pipette 100 μ L of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
 8. Cover the plate and incubate for 30 minutes on a plate shaker (approximately 200 rpm) at room temperature.
 9. Wash the wells again in the same manner as step 6.
 10. Pipette 150 μ L of LIA working substrate solution into each well (We recommend using a multichannel pipette).
 11. Measure the RLUs in each well on a microplate luminometer

CALCULATIONS

1. Calculate the mean RLU of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean RLUs on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean RLU of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 100 ng/mL then dilute it with calibrator A at a dilution of no more than 1:10. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA**

Sample data only. Do not use to calculate results.

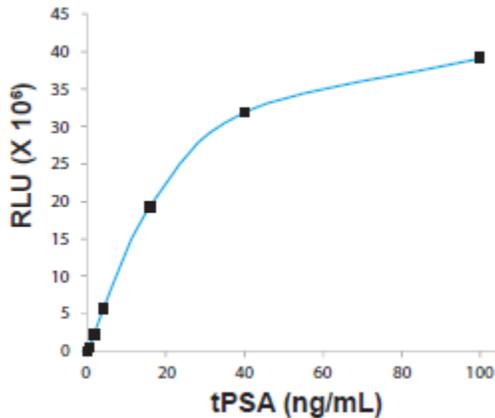
Calibrator	RLU 1 $\times 10^3$	RLU 2 $\times 10^3$	Mean RLU $\times 10^3$	RLU/RLU _{MAX} (%)
A, 0 ng/mL	3590	3920	3755	0.01
B, 0.1 ng/mL	20410	19380	19895	0.05
C, 0.4 ng/mL	312610	295950	304280	0.8
D, 1.6 ng/mL	1995810	1999240	1997525	5.3
E, 4 ng/mL	5339470	5527700	5433585	14.5
F, 16 ng/mL	18274310	18481930	18379120	49.0
G, 40 ng/mL	30455730	30389730	30422730	81.2



H, 100 ng/mL	37445530	37533250	37489390	100
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** It is recommended to use the RLU/RLU_{MAX} values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show quite different RLU values, however, the RLU/RLU_{MAX} values remain consistent.

TYPICAL CALIBRATOR CURVE



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of quantitation was calculated from the standard curve by determining the resulting concentration of the mean RLU of calibrator A (based on 40 replicate analyses) plus 3 SD. Therefore, the sensitivity of the total PSA LIA kit is **0.082 ng/mL**.

INTRA-ASSAY PRECISION

Three samples were assayed 20 times each on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.724	0.035	4.8
2	2.94	0.13	4.4
3	7.01	0.26	3.6

INTER-ASSAY PRECISION

Three samples were assayed 20 times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.75	0.05	6.6
2	2.87	0.22	7.8
3	6.41	0.56	8.8



RECOVERY

Spiked samples were prepared by adding defined amounts of PSA to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	1.8	-	-
+ 2.0	3.63	3.81	95.3
+ 8.0	9.01	9.81	91.8
+ 32.0	29.41	33.81	87.0
2 Unspiked	0.07	-	-
+ 2.0	2.05	2.07	99.0
+ 8.0	7.35	8.07	91.1
+ 32.0	28.42	32.07	88.6
3 Unspiked	0.005	-	-
+ 2.0	1.96	2.01	97.7
+ 8.0	6.94	8.01	86.7
+ 32.0	28.38	32.01	88.7

LINEARITY

Three patient serum samples were diluted with calibrator A (matrix). The results (in ng/mL) are tabulated below:

Sample: Matrix	Obs. Result	Exp. Result	Recovery %
1:0	64.4	-	-
4:1	50.6	51.5	98.2
3:2	37.0	38.7	95.7
2:3	24.6	25.8	95.4
1:4	12.8	12.9	99.0
1:10	6.86	6.44	106.5
1:20	3.7	3.21	114.9
1:0	40.2	-	-
4:1	31.8	32.1	99.0
3:2	23.6	24.1	98.1
2:3	16.2	16.1	100.5
1:4	8.05	8.03	100.2
1:10	4.61	4.02	114.8
1:20	2.33	2.01	116
1:0	43.1	-	-
4:1	33.0	34.5	95.7
3:2	24.1	25.9	93.0
2:3	16.6	17.2	96.0
1:4	8.84	8.62	102.6
1:10	4.89	4.31	113.5
1:20	2.47	2.16	114.6

COMPARATIVE STUDIES

The total PSA LIA kit (y) was compared with a leading competitor PSA immunoassay kit (x). The comparison of 40 serum samples yielded the following linear regression results:

$$y = 1.17x + 0.162, \quad r = 0.999$$



CROSS-REACTIVITY

Cross-reactivity was tested by spiking serum samples with kallikreins 6, 7, 8, 11 and 14 at concentrations up to 10 µg/mL (100 times the concentration of top calibrator). These proteins have structural similarities to PSA. No cross-reactivity was detected.

INTERFERENCE

Interference was tested by spiking serum samples, containing a range of PSA concentration levels, with Haemoglobin (up to 2g/L), bilirubin (up to 500 µM), triglycerides (up to 37 mM), HAS (up to 60 g/L) and combinations of them. Interference was less than 15% in all cases.

HIGH DOSE HOOK EFFECT

The total PSA LIA kit did not experience a high dose hook effect when it was tested up to a PSA concentration of 1000 ng/mL.

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. Serum samples from a group (n = 96) of apparently healthy males and females aged from 3-70 years old were assayed with the total PSA LIA Kit. Results are as follows:

Group	Range (ng/mL)
Males and Females (3-70 years old)	All <4.0

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.