



PSP94 Immunoassay for Urine Specimens

Catalog Number PSP94U0001

For the quantitative determination of prostate secretory protein
(also known as beta-microseminoprotein) in urine.

FOR RESEARCH USE ONLY: NOT FOR USE IN DIAGNOSTIC PROCEDURES

This Technical Summary sheet should be read thoroughly and completely before using this product.

Table of Contents

| | |
|--------------------------------------|----|
| PSP94 BACKGROUND..... | 3 |
| PRINCIPLE OF THE ASSAY | 3 |
| PROCEDURE LIMITATIONS | 3 |
| MATERIALS PROVIDED | 4 |
| STORAGE | 4 |
| OTHER SUPPLIES REQUIRED | 5 |
| SAMPLE HANDLING..... | 5 |
| SAMPLE PREPARATION | 5 |
| REAGENT PREPARATION | 5 |
| ASSAY PROCEDURE..... | 6 |
| ASSAY PROCEDURE SUMMARY | 7 |
| TECHNICAL HINTS | 8 |
| TYPICAL RESULTS FOR PSP94 ASSAY..... | 9 |
| Calculation of Results | 9 |
| Typical Calibration Curve..... | 9 |
| Precision..... | 11 |
| Dilution Linearity..... | 11 |
| Sensitivity | 12 |
| Sample Values | 12 |
| REFERENCES | 12 |

PSP94 BACKGROUND

Prostate Secretory Protein (PSP94), also known as beta-microseminoprotein, or inhibin-like protein, is a basic 94 amino acid protein with a MW of 10,704 . Despite a growing body of evidence linking PSP94 to the development and prognosis of prostate cancer it has not been approved for diagnostic use as a tool to assist in making informed clinical decisions^(1,2). PSP94 is generated from a 114 amino acid precursor whose DNA sequence is located on chromosome 10⁽³⁾. Purified PSP94 isolated from seminal fluid migrates between 13 – 16 KDa on a polyacrylamide gel⁽³⁾ and the difference in MW is not due to glycosylation but due to the basic nature of the protein⁽²⁾

PSP94 is found in high concentration in the epithelial cells of the prostate⁽⁴⁾. A 31 amino acid cleavage product of PSP94 found in seminal fluid demonstrates the ability to inhibit Follicle Stimulating Hormone (FSH) release⁽⁵⁾. PSP94 has been examined in serum and urine for its potential utility as a biomarker. Results from Kaighn, et al⁽⁶⁾ demonstrated that PSP94 was not detectable in PC-3 cell line from human prostatic carcinomas. Furthermore, PSP94 in urine was decreased in men with late stage tumors using 24 hr collection⁽⁷⁻⁹⁾. Recently, Miraculins identified a peak at 10750 M/Z by mass spectrometry that decreased in urine patients with prostate cancer, subsequently identified as PSP94. Miraculins studies have also suggested potential utility when combined with other biomarkers such as total Prostate Specific antigen (PSA) or Free/Total PSA.

PRINCIPLE OF THE ASSAY

This assay uses a quantitative sandwich enzyme immunoassay format. A polyclonal antibody specific for PSP94 has been coated onto 96 well ELISA plates and prepared with an overcoat solution for long term storage. Calibrators, controls or samples are then added to the plate. PSP94 present in urine is bound by the polyclonal antibody attached to the 96 well ELISA plate. After washing away unbound substances, a mouse anti-PSP94 horse radish peroxidase (HRPO) conjugate is added to each well. The wells are washed and a HRPO substrate is added. The color development is stopped and the color is proportional to the amount of PSP94 bound per well.

PROCEDURE LIMITATIONS

- FOR RESEARCH USE ONLY: NOT FOR USE IN DIAGNOSTIC PROCEDURES
- PSP94 ELISA Kit should not be used beyond expiration date
- Do not mix materials from different sources or lots

- Samples should be read within the range of the PSP94 ELISA kit. Samples above 6 ng/mL should be further diluted with 1X Assay Diluent. Samples below the detection limit of the assay should be run at higher concentration.
- The PSP94 ELISA is designed to be a rugged test. However, any variation in pipetting, buffer preparation, sample handling, washing technique, operator, incubation time can lead to increased variation in PSP94 quantitation.
- The sensitivity of the PSP94 ELISA allows for dilution of urine in 1X Assay Diluent at a recommended 1:100 dilution. These conditions were designed to minimize the interference of urine. Until all factors have been tested, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

| Part | Label | Material Description | amount | |
|------------|----------------------------------|---|--------|---------|
| N/A | N/A | Mylar Plate Sealers | 4 | Piece |
| MoM000135 | 25x Wash Concentrate | 25X Wash Buffer with ProClin 300 (20 mL) | 1 | vial |
| MoM0000145 | 5x Assay Buffer | 5X Assay Buffer with ProClin 300 (23 mL) | 1 | vial |
| MoM0000475 | PSP94 ELISA Plate | Precoated Plates - PSP94 (1 x 96 well plate) | 1 | plate * |
| MoM0000545 | Anti-PSP94 Conjugate Concentrate | PSP94 Conjugate Concentrate in stabilizers with ProClin 300 (200 µL) | 1 | vial |
| MoM0000555 | TMB Solution | TMB Solution (10 mL) | 1 | vial |
| MoM0000435 | 0.2N Stop Solution | 0.2N sulfuric acid stop solution (5 mL) | 1 | vial |
| MoM0000425 | PSP94 12ng/mL Calibrator | 12 ng/mL PSP94 Calibrator stock solution in stabilizers with ProClin (1 mL) | 1 | vial |

* Please contact us for partial plates in 4x24 well or 2x 48 well

STORAGE

Store unopened kits at 2-8°C. Do not use past expiration date, mentioned on the cover. All opened and diluted materials are stable at 2-8°C for up to 1 month provided that the final date is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 600 nm (range 540 – 600nm)
- Pipettes and pipette tips
- Deionized or distilled water
- 500 mL graduated cylinder
- Test tubes for dilution
- Reagent reservoirs

SAMPLE HANDLING

Urine - Aseptically collect midstream urine voided directly into a sterile container for a spot collection or collect urine over 24 hours in an appropriate container for a 24 hour urine collection. Vortex the sample for 3-5 seconds, then centrifuge the urine sample at 10,000g for 4 minutes to remove particulate matter. Collect the assay supernatant and test immediately or transfer supernatant to a fresh tube and store at less than -10° C or at 2-8°C dependent on length of storage time desired.

Note: PSP94 is stable at 2-8°C for 1 week and is stable to 2 freeze thaw cycles. For best results, minimize the number of freeze/thaw cycles.

When using frozen samples, thaw overnight at 2-8°C or thaw rapidly at room temperature, vortex the sample for 3-5 seconds then re-centrifuge and collect supernatant of the urine samples as indicated above.

Note: urine should not be collected after a digital rectal exam due to the high release of PSP94 from the prostate.

SAMPLE PREPARATION

Samples recommended dilution is 1:100 with 10 µL of urine + 990 µL of 1X Assay Diluent. This allows for the majority of the samples to be within the calibration. Samples that read higher than the 6 ng/mL calibrator should be further diluted, while samples lower than the detection limit should be run more concentrated.

REAGENT PREPARATION

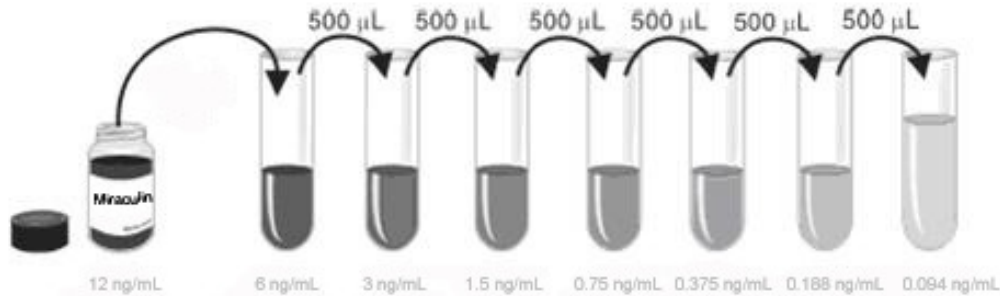
Note: Warm all kit reagents to room temperature prior to use.

25x Wash Concentrate – Dilute 20mL of 25x Wash Concentrate in 480mL of distilled or deionized water to prepare 500mL of Wash Buffer.

Note: If crystals have formed in the concentrate, warm to room temperature and agitate gently until crystals dissolve back into solution prior to performing dilutions.

5x Assay Buffer – Dilute 8mL of 5x Assay Buffer in 32mL of distilled or deionized water to prepare 40mL of 1X Assay Diluent.

PSP94 Standards – Prepare a standard curve by serially diluting the 12ng/mL PSP94 Calibrator supplied.



Pipette 500µL of Assay Diluent into 8 separate tubes (not supplied).

Pipette 500µL of the PSP94 12ng/mL Calibrator into the first tube and mix. Complete the serial dilution as outlined in the above diagram, transferring 500µL of the previous standard to the next standard each time.

Anti-PSP94 Conjugate Concentrate – Dilute 200µL of Anti-PSP94 Conjugate Concentrate in 19.8mL of 1X Assay Diluent to prepare 20mL of 1:100 diluted PSP94 Conjugate. Store at room temperature, wrapped in foil to protect it from light, until use.

ASSAY PROCEDURE

Note: Warm all kit reagents and urine samples to room temperature prior to use. It is recommended to assay all calibrators and samples in replicates.

1. Prepare all working reagents, standards and samples as directed in previous sections.
 - a. Prepare standard curve as outlined above.
 - b. Prepare urine samples by vortexing urine samples for 3-5 seconds then centrifuge at 10,000g for 4 minutes. Using the supernatant, perform a 1:100 dilution using 1X Assay Diluent as outlined in the Sample Preparation above.
2. Remove precoated PSP94 ELISA from the foil pouch.

3. Add 75 μ L of Assay Diluent to all wells.
4. Add 75 μ L of prepared standards and samples to the appropriate wells.
Note: Total volume in each well should now be 150 μ L.
5. Cover the plate with an adhesive sealer and shake on an orbital rotator set at 500 rpm for 1 minute.
6. Incubate the plate on an orbital rotator set at 200rpm for 2 hours.
7. Prepare working dilution of the Anti-PSP94 Conjugate Concentrate as outlined above.
8. Invert the plate or aspirate wells to remove liquid in all wells.
9. Using a multichannel, squirt bottle or automated washer, wash the plate three times by filling each well with 400 μ L/well of Wash Buffer and inverting. Complete removal of liquid after each wash is essential to good performance. After the last wash, ensure plate is adequately blotted on absorbent material.
10. Add 200 μ L/well of 1:100 diluted Anti-PSP94 Conjugate.
11. Cover the plate with an adhesive sealer and foil.
12. Incubate the plate on an orbital rotator set at 200rpm for 2 hours. **Protect from light.**
13. Repeat steps 8 and 9 for a total of 3 washes.
14. Add 100 μ L/well of TMB solution.
15. Incubate at room temperature for 30 minutes on the benchtop. **Protect from light.** The wells will turn blue in color; if the blue color is not uniform and concentrates around the edges of each well, gently tap the plate to encourage dispersal of the TMB solution in the wells.
16. Add 50 μ L/well of 0.2N Stop Solution. The color should turn from blue to yellow; if the well color is green or the color is not uniform, gently tap the plate to encourage mixing.
17. Read the plate on a microplate reader at 450nm. If wavelength correction is available, set it to 600nm. If wavelength correction is not available, read the plate at 600nm in addition to 450nm and manually subtract out the plate background.

ASSAY PROCEDURE SUMMARY

1. Prepare working reagents, standards and samples as instructed.



2. Add 75 μ L of Assay Diluent to all wells. Followed by the addition of 75 μ L of prepare calibrator or sample to the appropriate wells.



3. Aspirate and wash 3 times.



4. Add 200 μ L of 1:100 diluted Anti-PSP94 Conjugate to all wells. Incubate for 2 hours on the shaker at RT. Protect from light.



5. Aspirate and wash 3 times.



6. Add 100 μ L of TMB Solution to all wells. Incubate for 30 minutes on the bench top. Protect from Light.



7. Add 50 μ L 0.2N Stop Solution to all wells.



8. Read plate at 450 nm with correction at 600nm.

TECHNICAL HINTS

- When diluting 5X Assay Buffer, Anti-PSP94 Conjugate Concentrate or PSP94 12ng/mL Calibrator, always avoid foaming.
- Change pipette tips between additions of each standard level, between sample additions, and between reagent additions.
- Use separate reservoirs for each reagent.
- Keep the plate flat at all times to avoid cross contamination between wells.
- To ensure accurate results, use provided plate sealers during incubation steps.
- Keep TMB Solution protected from light. Increasing calibrator concentration should be colorless for 0 ng/mL calibrator and increase in blue color with increasing calibrator concentration.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color will turn from blue to yellow after addition to the wells. A green color indicates that the Stop Solution has not mixed thoroughly with the Substrate Solution.

TYPICAL RESULTS FOR PSP94 ASSAY

Results of a typical PSP94 ELISA are shown below. Any variation in the standards, diluents, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples *are for the purpose of illustration only*, and should not be used to calculate unknowns. Each user should obtain their own standard curve.

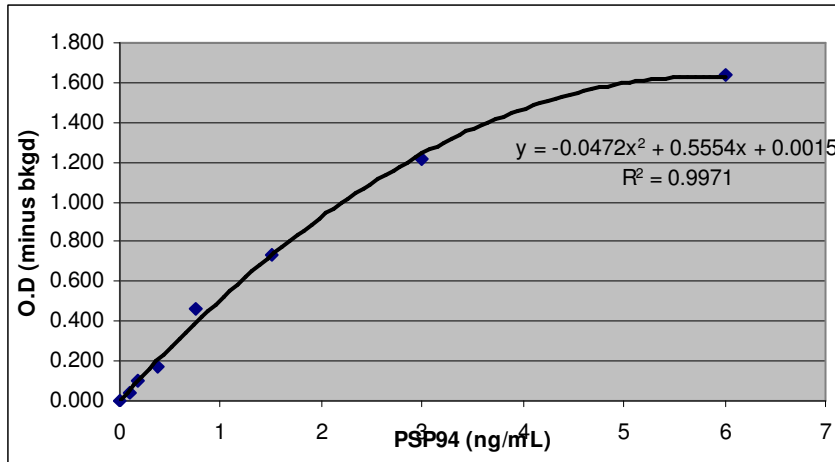
Calculation of Results

The average of duplicate readings for each calibrator, control and sample are calculated and subtract the zero standard optical density. One can use software packages to generate a 4-PLC curve fit, 2nd order polynomial fit or log/log plot for quantitation of PSP94 by plotting the mean absorbance for each calibrator on the y-axis against the PSP94 concentration on the x-axis. Sample concentrations are quantitated from the standard curve and then multiplying by the dilution factor

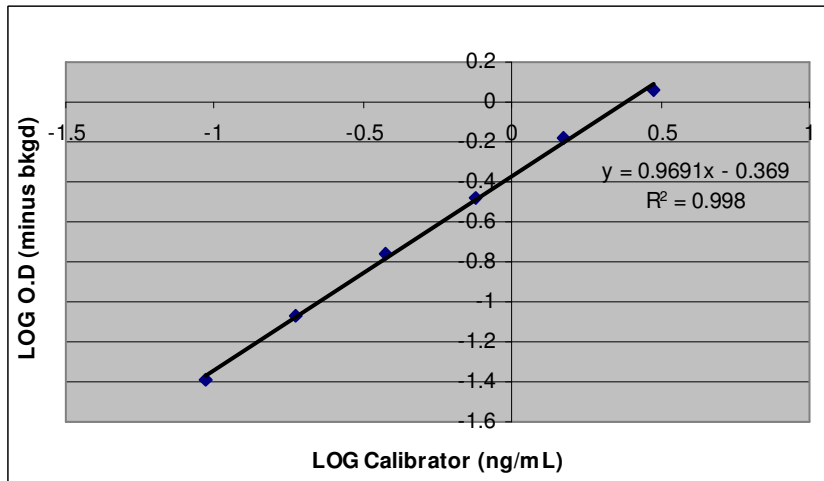
Typical Calibration Curve

| Calibrator | OD | OD corrected |
|------------|-------|--------------|
| 6 | 1.663 | 1.630 |
| | 1.722 | 1.689 |
| 3.000 | 1.161 | 1.128 |
| | 1.203 | 1.170 |
| 1.500 | 0.694 | 0.661 |
| | 0.688 | 0.655 |
| 0.750 | 0.369 | 0.336 |
| | 0.366 | 0.333 |
| 0.375 | 0.213 | 0.180 |
| | 0.204 | 0.171 |
| 0.188 | 0.115 | 0.082 |
| | 0.121 | 0.088 |
| 0.094 | 0.076 | 0.043 |
| | 0.071 | 0.038 |
| 0 | 0.035 | |
| | 0.031 | |

The calibration curve below represents a 2nd order polynomial curve fit.



The calibration curve below represents a Log / Log Plot with a PSP94 range of 0.094 to 3.0 ng/mL.



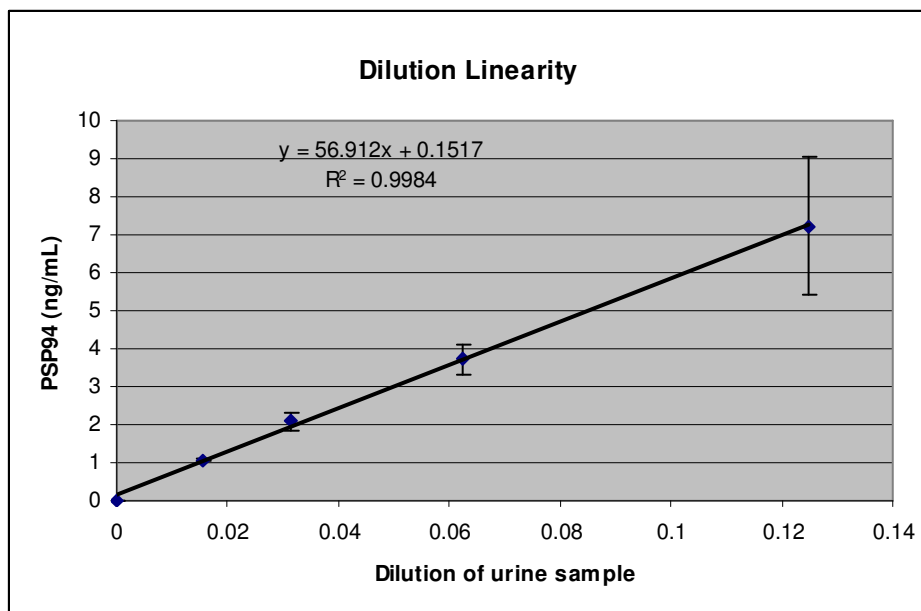
Precision

The table below represents 16 replicates of a Low, Medium and High sample that were diluted 1:100 and then tested in duplicate from 8 calibration curves. Each calibration curve was run with independently prepared calibrator to simulate actual customer running conditions and each sample was quantitated from its calibration curve.

| Sample | Within Run Imprecision | | | Between Run Imprecision | | Total Imprecision %CV |
|--------|------------------------|------|------|-------------------------|------|-----------------------|
| | Mean (ng/mL) | SD | %CV | SD | %CV | |
| Low | 48.4 | 2.11 | 4.3% | 2.60 | 5.4% | 6.9% |
| Medium | 146.8 | 4.23 | 2.9% | 8.01 | 5.5% | 6.2% |
| High | 228.4 | 7.98 | 3.5% | 9.29 | 4.1% | 5.4% |

Dilution Linearity

Below is an example of a sample that was serial diluted from 1: 4 to 1:64 with 1X Assay Buffer. The error bars represent the standard deviation of duplicates. The +/- 2 ng/mL SD at 1:4 (0.125) is due to the sample being at the high end of the calibration curve. Thus, we recommend a calibration curve starting from 6 ng/mL for optimum quantitation.



Sensitivity

The median detection limit for 8 calibration curves run in duplicate is 16 pg/mL.

Sample Values

In early research studies by Teni et al in 1988, an average PSP94 value for normal male urine was 120 ug/24 hr collection and 24 ug/24 hr collection for individuals with prostate cancer.⁶ Based on these early research results, typical values for PSP94 in urine for a 1.0 Liter collection in 24 hours could be considered to be 120 ng/mL (120,000 ng/1000 mL) for men without prostate cancer and 24 ng/mL for men with prostate cancer.

REFERENCES

1. Whitaker HC, Warren AY, Eeles R, Kote-Jarai Z, Neal DE. The potential value of microseminoprotein-beta as a prostate cancer biomarker and therapeutic target. *Prostate* 2010; 70:333-40.
2. Seidah NG, Arbatti NJ, Rochemont J, Sheth AR, Chretien M. Complete amino acid sequence of human seminal plasma beta-inhibin. Prediction of post Gln-Arg cleavage as a maturation site. *FEBS Lett* 1984; 175:349-55.
3. Dube JY, Frenette G, Paquin R, et al. Isolation from human seminal plasma of an abundant 16-kDa protein originating from the prostate, its identification with a 94-residue peptide originally described as beta-inhibin. *J Androl* 1987; 8:182-9.
4. Brar A, Mbikay M, Sirois F, Fournier S, Seidah NG, Chretien M. Localization of the human prostatic secretory protein PSP94 and its mRNA in the epithelial cells of the prostate. *J Androl* 1988; 9:253-60.
5. Ramasharma K, Sairam MR, Seidah NG, et al. Isolation, structure, and synthesis of a human seminal plasma peptide with inhibin-like activity. *Science* 1984; 223:1199-202.

6. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Invest Urol* 1979; 17:16-23.
7. Teni TR, Sheth AR, Kamath MR, Sheth NA. Serum and urinary prostatic inhibin-like peptide in benign prostatic hyperplasia and carcinoma of prostate. *Cancer Lett* 1988; 43:9-14.
8. Teni TR, Bandivdekar AH, Sheth AR, Sheth NA. Prostatic inhibin-like peptide quantified in urine of prostatic cancer patients by enzyme-linked immunosorbent assay. *Clin Chem* 1989; 35:1376-9.
9. Tremblay J, Frenette G, Tremblay RR, Dupont A, Thabet M, Dube JY. Excretion of three major prostatic secretory proteins in the urine of normal men and patients with benign prostatic hypertrophy or prostate cancer. *Prostate* 1987; 10:235-43.