Prostate Specific Antigen (PSA) Activity Assay Kit (Fluorometric) (#BN01122)

(Catalog # BN01122; 100 assays, Store kit at -20°C)

I. Introduction:

Prostate Specific Antigen (PSA, EC 3.4.21.77), or Kallikrein-related peptidase 3, is a serine protease. It proteolyses the Semenogelin I and Semenogelin II of seminal fluid and thus liquefies it. PSA also shows antiangiogenic activity. The significance of the level of enzymatically active PSA is emerging in diagnosis and prognosis of prostate cancer. Growing number of evidence suggests that PSA plays an important role in prostate tumor growth, invasion and metastasis. Assay Genie's PSA Activity Assay Kit enables the research in this field. This kit utilizes the ability of an active PSA to cleave a synthetic AMC based peptide substrate to release a free fluorophore. The released AMC can be easily quantified using a fluorescent microplate reader. This assay kit is simple and can be used in a high-throughput format. This assay can detect as low as 1 ng of purified active PSA.

PSA Substrate-AMC $\xrightarrow{\text{PSA}}$ Cleaved PSA Substrate + AMC **(Ex/Em= 360/460 nm)**

II. Applications:

- Detection of PSA activity in seminal fluid
- Determination of enzymatic activity of purified PSA

III. Sample Type:

- Seminal fluid
- Purified PSA

IV. Kit Contents:

Components	BN01122	Cap Code	Part Number
PSA Assay Buffer	25 ml	WM	BN01122-1
PSA Dilution Buffer	1.8 ml	Clear	BN01122-2
PSA Positive control	10 µl	Green	BN01122-3
PSA Substrate (10 mM)	400 µl	Brown	BN01122-4
AMC-Standard (1 mM)	100 µl	Yellow	BN01122-5

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well fluorescence microplate reader.

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the assay.

- **PSA Assay Buffer** and **PSA Dilution Buffer**: Ready to use. Store at 4 °C or -20 °C. Bring to room temperature before use.
- **PSA Positive Control**: Store at -20°C. Avoid multiple freeze/thaw of the enzyme. Use within 3 months.
- **PSA Substrate**: Ready to use. Store at -20 °C. Thaw before use.
- **AMC Standard**: Store at -20°C.

VII. PSA Activity Assay Protocol:

1. **Sample Preparation:** Dilute seminal fluid ~100X with PSA Assay Buffer. Then, use this diluted seminal fluid directly in your experiment. For Sample (S), add 1-10 µl of diluted seminal fluid into desired well(s) in a white 96-well plate. If necessary, dilute the seminal fluid with PSA Assay buffer. For Background Control (BC), add assay buffer. For Positive control (PC), before use, thaw and then dilute by adding 90 µl PSA Dilution Buffer. Add 10 µl of the PSA Positive Control into desired well(s). Adjust the volume of S, BC, and PC to 50 µl/well with PSA Assay Buffer.
2. **AMC Standard Curve Preparation:** Thaw and then prepare 150 µM solution of AMC-Standard by diluting 15 µl of 1 mM AMC-Standard with 85 µl of PSA Assay Buffer. Add 0, 2, 4, 6, 8 and 10 µl of 150 µM AMC-Standard into a series of wells in a 96-well plate and adjust the final volume to 100 µl/well with PSA Assay Buffer to generate 0, 300, 600, 900, 1200 and 1500 pmol/well of AMC Standard respectively. Mix well and measure the fluorescence (Ex/Em 360/460 nm) in end point mode.

   Note: For unknown samples, we suggest testing several dilutions to ensure the readings are within the Standard Curve range.
3. **PSA Substrate Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Substrate Mix:

   46 µl PSA Assay Buffer
   4 µl PSA Substrate

   Mix & add 50 µl of PSA Substrate Mix into each of S, BC, and PC/ PSA Positive Control. Mix well.

   Note: Don't add Substrate Mix to the AMC Standard wells.
4. **Measurement:** Measure fluorescence (Ex/Em 360/460 nm) in a kinetic mode for 30 mins -1 hr at 37°C. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2). Calculate $\Delta RFU/\Delta t$.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the AMC-Standard Curve and obtain the slope of the curve (ΔRFU/pmol). If substrate background control reading is significant then subtract the background control reading from sample reading. To calculate the specific PSA activity of sample, subtract ΔRFU of Negative Control (ΔRFU_{NC}) from Sample (ΔRFU_S).

$$\textbf{Sample PSA Activity} = \textbf{B X D}/(\textbf{Δt} * \mu\textbf{l of Seminal fluid}) = \textbf{pmol}/\textbf{min}/\mu\textbf{l} = \textbf{mU}/\mu\textbf{l}$$

Where:

B = Released MCA in sample based on Std. curve slope (pmol)
Δt = Reaction time (min.)
D = Sample dilution factor (D=1 when samples are undiluted)

Unit Definition: One unit of PSA activity is the amount of enzyme that catalyzes the release of 1 nmol of AMC per min from the substrate under the assay conditions at room temperature.

To measure the amount of PSA in a seminal fluid sample:

Apply sample's ΔRFU to AMC Standard Curve to obtain corresponding AMC (in pmole) and calculate the concentration of PSA in the sample in ng/µl as.

$$[PSA] = \frac{B}{\Delta t \times 0.2 \times \mu l \ of \ Seminal \ fluid} * D = ng/\mu l$$

Where, 0.2 is the conversion factor for amount of AMC released under the same assay conditions to fully active PSA.

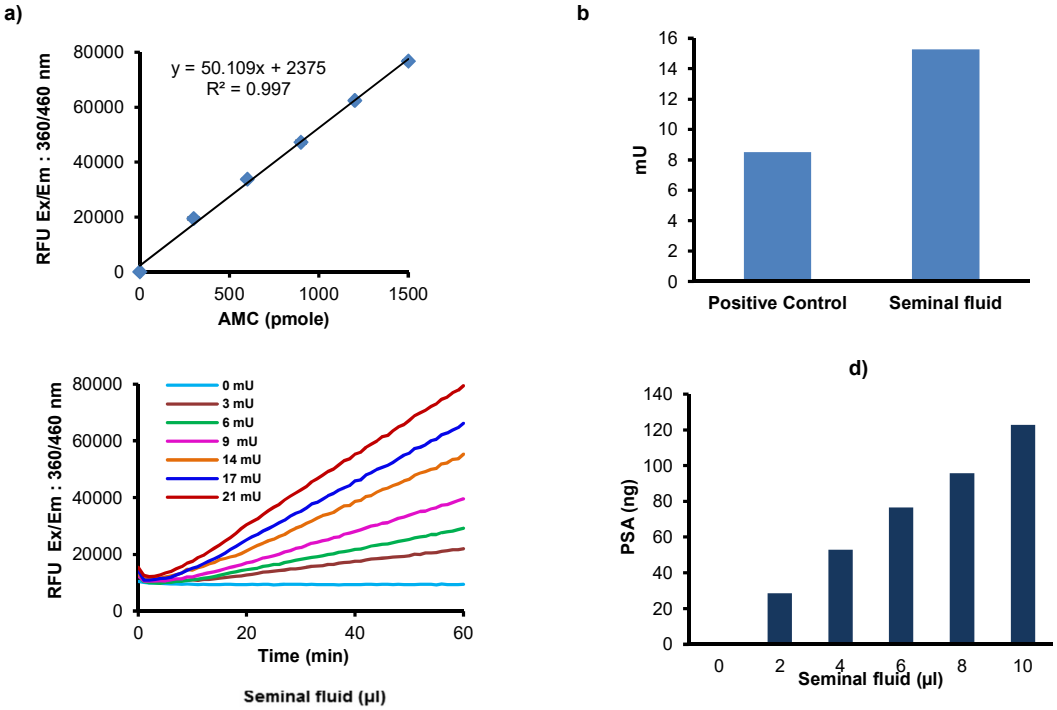

Figure: (a) **AMC -Standard Curve** (300-1500 pmol), error bars indicate SD (n=3). (b) **PSA activity**: In Positive Control and in 100x diluted (6 µl) seminal fluid. (c) **Kinetic activity curves** using different amounts of PSA Positive Control in the assay. d) **PSA in** seminal fluid: Amount of PSA in ng in 2 to 10 µl of 100x diluted seminal fluid samples.

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