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ab273302 Prostate Specific Antigen (PSA) Activity Assay Kit (Fluorometric)

View Kit datasheet: <https://www.abcam.com/ab273302>
(use <https://www.abcam.cn/ab273302> for china, or
<https://www.abcam.co.jp/ab273302> for Japan)

For the determination of Prostate Specific Antigen (PSA) Activity in Seminal fluid and purified PSA.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Prostate Specific Antigen (PSA) Activity Assay Kit (Fluorometric) (ab273302) 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.

2. Protocol Summary

Prepare samples and positive control as directed



Prepare all reagents as directed



Prepare standard curve and measure fluorescence at Ex/Em = 360/460 nm. Calculate slope



Add Positive Control, Samples, Background Control to appropriate wells and adjust volume to 50 μ L



Add PSA Substrate Mix(50 μ L)



Measure fluorescence (Ex/Em= 360/460 nm) in a kinetic mode for 30 min – 1 hour at 37°C.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 3 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
PSA Assay Buffer	25 mL	-20°C or 4°C
PSA Dilution Buffer	1.8 mL	-20°C or 4°C
PSA Positive Control	10 µL	-20°C
PSA Substrate (10 mM)	400 µL	-20°C
AMC Standard	100 µL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microplate reader capable of measuring fluorescence at Ex/Em = 360/460 nm
- 96-well black plate with flat bottom

8. Technical Hints

- **This kit is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 PSA Assay Buffer:

Ready to use. Bring to room temperature before use.
Store at 4 °C or -20 °C.

9.2 PSA Dilution Buffer:

Ready to use. Bring to room temperature before use.
Store at 4 °C or -20 °C.

9.3 PSA Positive Control:

Store at -20°C.
Avoid multiple freeze/thaw of the enzyme.
Use within 3 months.

9.4 PSA Substrate:

Ready to use. Thaw before use.
Store at -20 °C.

9.5 AMC Standard:

Store at -20°C.

10. Sample Preparation

10.1 Seminal fluid preparation:

10.1.1 Dilute seminal fluid ~100X with PSA Assay Buffer.

Δ Note: For unknown samples, we suggest testing several dilutions to ensure the readings are within the Standard Curve range.

10.2 Positive Control:

10.2.1 Before use, thaw and then dilute by adding 90 µl PSA Dilution Buffer.

11. Standard Curve

Thaw all reagents thoroughly and mix gently.

- 11.1 Prepare 150 μM solution of AMC-Standard by diluting 15 μl of 1 mM AMC Standard with 85 μl of PSA Assay Buffer.
- 11.2 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. This will generate 0, 300, 600, 900, 1200 and 1500 pmol/well of AMC Standard respectively.

Standard #	150 μM AMC Standard (μL)	PSA Assay Buffer (μL)	AMC (pmol/well)
1	0	100	0
2	2	98	300
3	4	96	600
4	6	94	900
5	8	92	1200
6	10	90	1500

- 11.3 Mix well and measure the fluorescence (Ex/Em 360/460 nm) in end point mode.

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

- 12.1.1 For Sample, add 1-10 μl of diluted seminal fluid into desired wells in a white 96-well plate.
- 12.1.2 For Background Control, add assay buffer.
- 12.1.3 For Positive control, add 10 μl of the diluted PSA Positive Control into desired wells.
- 12.1.4 Adjust all volumes to 50 μl /well with PSA Assay Buffer.

PSA Substrate Mix:

- 12.1.5 Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 μl of the Mix:

Component	Reaction Mix (μL)
PSA Assay Buffer	46
PSA Substrate	4

- 12.1.6 Add 50 μl of Reaction Mix to each wells and mix well.

Δ Note: Don't add Substrate Mix to the AMC Standard wells.

- 12.1.7 Measure fluorescence (Ex/Em 360/460 nm) in a kinetic mode for 30 mins -1 hr at 37°C.
- 12.1.8 Choose two time points (t1 & t2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2).
- 12.1.9 Calculate $\Delta\text{RFU}/\Delta t$.

13. Calculations

13.1 Subtract 0 Standard reading from all readings.

13.2 Plot the AMC-Standard Curve and obtain the slope of the curve ($\Delta\text{RFU}/\text{pmol}$).

Δ Note: If substrate background control reading is significant then subtract the background control reading from sample reading.

13.3 To calculate the specific PSA activity of sample, subtract ΔRFU of Negative Control (ΔRFUNC) from Sample (ΔRFUS).

$$\text{Sample PSA activity} = \frac{B}{(\Delta t \times \mu\text{L seminal fluid})} \times D \quad (\text{pmol}/\text{min}/\mu\text{L})$$

B = Released MCA in sample based on Std. curve slope (pmol)

Δt = Reaction time (min)

D = Dilution factor (D = 1 for undiluted Samples)

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:

13.4 Apply sample's ΔRFU to AMC Standard Curve to obtain corresponding AMC (in pmole).

13.5 Calculate the concentration of PSA in the sample in ng/ μL as:

$$\text{PSA} = \frac{B}{(\Delta t \times 0.2 \times \mu\text{L seminal fluid})} \times D \quad (\text{ng}/\mu\text{L})$$

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

14. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

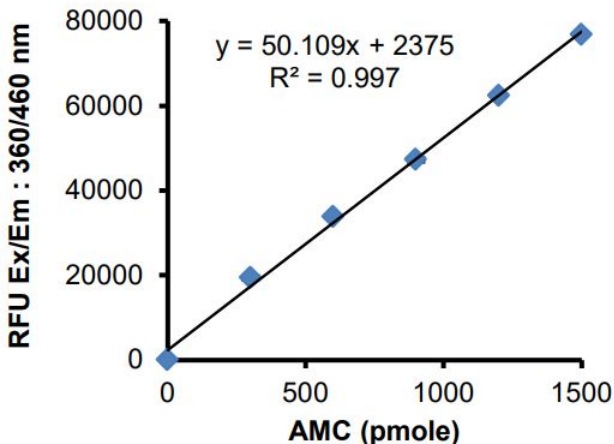


Figure 1. AMC Standard Curve (300-1500 pmol), error bars indicate SD (n=3).

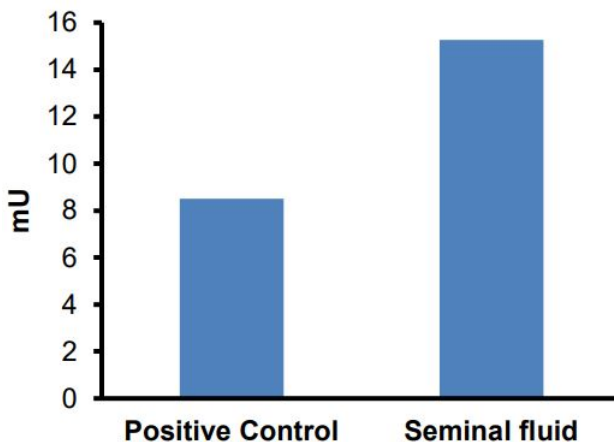


Figure 2. PSA activity: In Positive Control and in 100x diluted (6 μ l) seminal fluid.

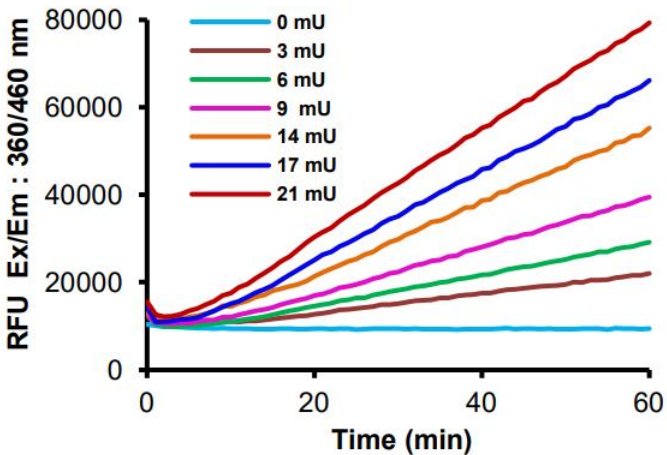


Figure 3. Kinetic activity curves using different amounts of PSA Positive Control in the assay.

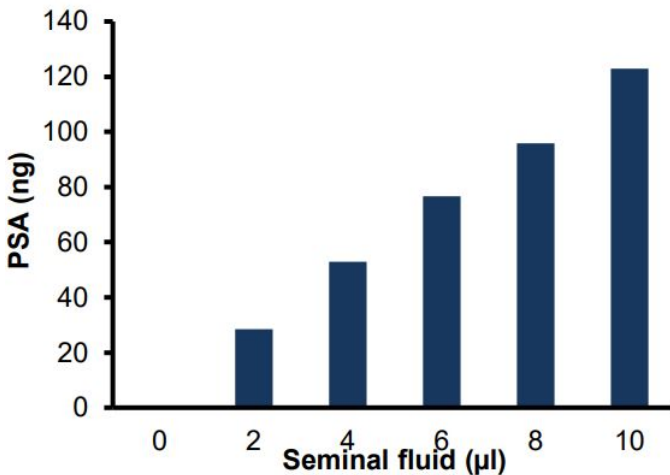


Figure 4. PSA in seminal fluid: Amount of PSA in ng in 2 to 10 µl of 100x diluted seminal fluid samples.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

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