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Product Information

Plasmin Activity Assay Kit (Fluorometric)

Catalog Number **MAK244**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Plasmin (EC 3.4.21.7) is a serine protease occurring in plasma as plasminogen. Upon activation via cleavage by plasminogen activators, plasmin solubilizes fibrin clots and activates and/or degrades compounds of the coagulation and complement systems. The plasminogen-plasmin system has also been implicated in a wide variety of physiologic and pathologic processes, including tumor growth, invasion, and metastasis.

The Plasmin Activity Assay Kit is based on the ability of plasmin to proteolytically cleave a synthetic substrate and release a fluorophore, AMC, which can be easily quantified by fluorescence microplate readers. This assay kit is simple, rapid, and can detect plasmin activity as low as 10 ng in a variety of samples.

Components
The kit is sufficient for 100 assays in 96 well plates.

Plasmin Assay Buffer Catalog Number MAK244A	15 mL
Plasmin Dilution Buffer Catalog Number MAK244B	1.5 mL
Plasmin Enzyme Standard (1 mg/mL) Catalog Number MAK244C	5 µL
Plasmin Substrate Catalog Number MAK244D	0.2 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – white plates are preferred for this assay.
- Fluorescence multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge small vials at low speed prior to opening.

Plasmin Assay Buffer – Bring to room temperature before use.

Plasmin Enzyme Standard – Aliquot and store at –80 °C. Avoid repeated freeze/thaw cycles.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate. Read entire protocol before performing the assay.

Sample Preparation
Add 2–50 µL of sample containing plasmin per well of 96 well plate and adjust the volume to 50 µL with Plasmin Assay Buffer.

Note: (Optional) For samples having fluorescence background, prepare in parallel sample background control well(s) containing sample only and adjust the volume to 100 µL/well with Plasmin Assay Buffer.

Standard Curve Preparation

Prepare working solution of Plasmin Enzyme working solution (10 ng/μL) by adding 198 μL of Plasmin Dilution Buffer to 2 μL of Plasmin Enzyme Standard. Mix well by pipetting up and down.

Add 0, 5, 10, 15, 20, and 25 μL of Plasmin Enzyme working solution (10 ng/μL) into a series of wells in a 96 well plate to prepare 50, 100, 150, 200, and 250 ng/well of Plasmin Enzyme Standard.

Adjust the volume to 50 μL/well with Plasmin Assay Buffer.

Note: The unused Plasmin Enzyme working solution may be stored at –20 °C for two weeks or –80 °C for up to 2 months.

Plasmin Substrate Mix

Prepare enough Substrate Mix for the number of assays to be performed. Prepare 50 μL of Substrate Mix for Standard and sample wells, see Table 1.

Table1.
Preparation of Substrate Mix

Reagent	**Volume**
Plasmin Assay Buffer	48 μL
Plasmin Substrate	2 μL

Mix and add 50 μL of Plasmin Substrate Mix into Standard and sample well(s). Mix well.

Measurement

Measure fluorescence in kinetic mode for 10–20 minutes at 37 °C (λ_{ex} = 360 nm/λ_{em} = 450 nm). Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2).

Results

Calculations

Subtract 0 Standard reading from all readings. Plot the Plasmin Standard Curve.

Compare ΔRFU of the sample to the Plasmin Standard Curve to obtain corresponding Plasmin amount (B, in ng) and calculate the activity of Plasmin in the sample as:

$$\text{Plasmin Activity} = \frac{B}{V} \times \text{dilution factor}$$

(ng/mL) (μg/L)

B = Plasmin amount from Standard Curve (ng)
V = The sample volume added into the reaction well (mL)

Note: If the sample background control reading is significant, subtract the sample background control reading from sample reading.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	White plates are preferred for this assay.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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