3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Plasmin Inhibitor Screening Kit (Fluorometric)

Catalog Number **MAK245**
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. Plasmin inhibitors are critical in the treatment of hyperfibrinolysis-associated blood loss and related complications.

This Plasmin Inhibitor Screening Kit utilizes the ability of plasmin to cleave a synthetic AMC-based peptide substrate and release a fluorophore, AMC, which can be easily quantified by fluorescence microplate readers. In the presence of plasmin specific inhibitors, the extent of cleavage reaction is reduced or completely abolished. The loss in the fluorescence intensity can be correlated to the amount of inhibitor present in the assay solution. The kit provides a simple and rapid method to screen potential inhibitors of Plasmin.

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

Plasmin Assay Buffer Catalog Number MAK245A	15 mL
Plasmin Dilution Buffer Catalog Number MAK245B	1.5 mL
Plasmin Enzyme Catalog Number MAK245C	15 µL
Plasmin Substrate Catalog Number MAK245D	0.2 mL
Plasmin Inhibitor (Aprotinin, 0.6 mM) Catalog Number MAK245E	0.1 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 – Aliquot the stock solution and store at –80 °C. Avoid repeated freeze/thaw cycles.

Storage/Stability
Store the kit at –20 °C, protected from light.

Procedure
Read entire protocol before performing the assay.

Enzyme Solution Preparation
Dilute Plasmin Enzyme 1:100 with Plasmin Dilution Buffer. Make as per the assay requirement. Mix well by pipetting up and down.

Mix enough Enzyme Solution for the number of assays to be performed, see Table 1. For each well, prepare 50 µL of Plasmin enzyme solution.

Table 1.
Preparation of Enzyme Solution

Reagent	Volume
Plasmin Assay Buffer	35 µL
Diluted Plasmin Enzyme	15 µL

Mix and add 50 µL of Plasmin Enzyme Solution into desired wells.

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

Screening compounds, Inhibitor Control, and Enzyme Control Preparations
Dissolve candidate inhibitors into proper solvent. Dilute to 10× the desired test concentration with Plasmin Assay Buffer. Add 10 µL of diluted test inhibitors (I) or Plasmin Assay Buffer (Enzyme Control, EC) into Plasmin Enzyme containing wells. As an Inhibitor Control (IC), add 1 µL of Plasmin Inhibitor and 9 µL of Plasmin Assay Buffer to Plasmin Enzyme well(s). Incubate at room temperature for 10–15 minutes.

Plasmin Substrate Preparation
For each well, prepare 40 µL of substrate solution, see Table 2.

Table 2.
Preparation of Substrate Solution

Reagent	Volume
Plasmin Assay Buffer	38 µL
Plasmin Substrate	2 µL

Mix and add 40 µL of Plasmin Substrate solution into each well. 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
Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the net ΔRFU (RFU_2–RFU_1) values with the time ΔT (T_2–T_1).

% Relative Inhibition = [Slope(EC)–Slope(S)]/Slope(EC) × 100

Note: Irreversible inhibitors that inhibit the plasmin activity completely at the tested concentration will have ΔRFU = 0 and will show 100% Relative Inhibition.

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

SJ,MAM 04/16-1