

SPECTROZYME® PL

REF 251L

Description

A chromogenic substrate for the amidolytic assay of plasmin and for reactions in which plasmin is generated or consumed.

Formula: H-D-Nle-CHA-Lys-pNA.2AcOH

Molecular Weight: 652.8

Chemical Name: H-D-norleucyl-cyclohexylalanyl-lysine-para-nitroanilide diacetate salt

Composition: Enzymatically digestible substrate colyophilized with glycine

Extinction Coefficient: $\epsilon_{405\text{ nm}} = 9650 \text{ M}^{-1} \cdot \text{cm}^{-1}$

Purity: < 0.5% free para-nitroaniline

Solubility: $\geq 10 \text{ mM}$ in deionized water
 $\geq 25 \text{ mg/mL}$ in methanol:water (1:9)

Assay Conditions/Substrate Kinetics

Enzyme activity is determined by measuring the increase in absorbance of the free chromophore (pNA) generated per unit time at a wavelength of 405 nm. At excess substrate concentrations, the rate at which the absorbance increases due to the amount of chromophore released is linearly related to enzyme concentration. Measurement can be made either through acid quenching of the reaction (end-point method), or through use of a kinetic recording spectrophotometer (initial-rate-method).

Under the following reaction conditions, the following substrate kinetics were found,

Buffer: 20 mM Tris, 200 mM NaCl, 0.1% PEG 8000, pH 7.4

Temperature: 37°C

Substrate: 1.0 mM REF 251L

Enzyme: 2 nM Human Plasmin (SD REF 411)

Kinetics: K_m : 35.8 μM K_{cat} : 16.8 sec^{-1}

Presentation

An amber glass, screw-capped vial containing 50 μmoles of lyophilized substrate.

Reconstitution

Reconstitute with filtered deionized water to create a stock solution with a concentration of 5.0 mM. The typical working range concentration is 0.5 – 1.0 mM.

Storage and Stability

Lyophilized substrate may be stored in the dark at room temperature up to the expiration date stated on the vial. Protect from moisture by allowing vial to reach room temperature prior to opening.

Reconstituted substrate may be stored in the dark for 1 week at room temperature, 2 months at 2° - 8°C, or for up to 6 months frozen at -20°C (Aliquot and freeze. Do not submit to freeze-thaw cycles).

Warnings and Precautions

For Research Use Only.

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Warning

Hazard Statements:	H315 Causes skin irritation.
	H319 Causes serious eye irritation.
	H335 May cause respiratory irritation.
Precautionary Statements:	P261 Avoid breathing dust.
	P264 Wash thoroughly after handling.
	P280 Wear protective gloves/eye protection.
	P337 + P313 If eye irritation persists: Get medical advice/attention.
	P403 + P233 Store in a well-ventilated place. Keep container tightly closed.

References

1. Gardell, S. J., *et al.* *Journal of Biological Chemistry* 1989, **30**: 17947-17952.
2. Wojta, J., *et al.* *Thrombosis and Haemostasis* 1989, **61**: 289-293.
3. Sheng, S., *et al.* *Proc. Natl. Acad. Sci. USA* 1998, **95**: 499-504.
4. Kassam, G., *et al.* *Journal of Biological Chemistry* 1998, **273**: 4790-4799.
5. Higazi, A. A., *et al.* *Journal of Biological Chemistry* 1995, **270**: 17375-17380.
6. Filipenko, N. R. and Waisman, D. M. *Journal of Biological Chemistry* 2000, **275**: 38877-38884.

Definition of Symbols

	Consult instructions for use
	Manufacturer
	Refer to Safety Data Sheet
	Contains...
	Temperature Limitation
	Lot Number
	Expiration Date
	Catalog Number