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

Formula: \( \text{H-D-Nle-CHA-Lys-pNA.2AcOH} \)
Molecular Weight: 652.8
Chemical Name: \( \text{H-D-norleucyl-cyclohexylalanyl-lysine-para-nitroanilide diacetate salt} \)
Composition: Enzymatically digestible substrate colyophilized with glycine
Extinction Coefficient: \( \varepsilon_{405 \text{ nm}} = 9650 \text{ M}^{-1} \cdot \text{cm}^{-1} \)
Purity: < 0.5% free para-nitroaniline
Solubility: ≥ 10 mM in deionized water

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: \( K_m : 35.8 \text{ M} \quad K_{cat} : 16.8 \text{ sec}^{-1} \)

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 Reseach Use Only.

| CONT | H-D-norleucyl-cyclohexylalanyl-lysine-para-nitroanilide diacetate salt |

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

Definition of Symbols

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