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BIOMEDICA DIAGNOSTICS

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SPECTROZYME® PCa

REF 336

Description

A chromogenic substrate for the measurement of Activated Protein C.

Formula:	H-D-Lys (γ-Cbo)-Pro-Arg-pNA.2AcOH
Molecular Weight:	773.8
Chemical Name:	H-D-(γ-carbobenzoxyl)-lysyl-prolyl-arginine-paranitroanilide diacetate salt
Composition:	Enzymatically digestible substrate colyophilized with glycine excipient
Extinction Coefficient:	$\varepsilon_{405\ nm}$ = 9650 $M^{-1} \cdot cm^{-1}$
Purity:	≤ 0.5% free para-nitroaniline
Solubility:	> 10 mM in distilled/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 $\lambda_{405\ nm}$. 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:	50 mM Tris-Imidazole, 150 mM NaCl, pH 8.4
Temperature:	37°C
Substrate:	4.0 mM REF 336
Enzyme:	Activated Protein C
Kinetics:	K_m: 0.303 mM V_{max}: 25.0 µmoles/min/mL enzyme

Suggested Assay Procedure

1. Add 50 µL of citrated plasma + 100 µL of Protac® (REF 245) at 0.5 units/mL.
2. Incubate for 5 minutes at 37°C.
3. Add 1.65 mL of buffer + 200 µL of SPECTROZYME PCa.
4. Measure the ΔOD/min using a spectrophotometer set at a wavelength of 405 nm.

Presentation

Amber glass vial containing 10 µmoles (7.74 mg) of lyophilized substrate.

Reconstitution

Reconstitute with 1 – 2 mL of filtered distilled/deionized water (not buffer) to create a stock solution with a concentration of 5.0 – 10.0 mM. The typical working range concentration is 4.0 mM.

Storage and Stability

Lyophilized substrate may be stored in the dark at 2°– 8°C up to the expiration date stated on the vial.

Reconstituted substrate may be stored 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, protected from light. Do not submit to multiple freeze-thaw cycles.

Warnings and Precautions

For Reseach Use Only.

CONT	H-D-(γ-carbobenzoxyl)-lysyl-prolyl-arginine-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.

Related Products

REF 245, Protac®, Direct Protein C Plasma Activator

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References

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2. Madden, R. M., *et al. Thrombosis Research* 1990, **57**: 425-435.
3. Cappelle, M., *et al. Proceeds of the National Academy of Science, USA* 1995, **92**: 6152-6156.
4. Regan, L. M., *et al. Journal of Biological Chemistry* 1996, **271**: 17499-17503.
5. Stearns-Kurosawa, D. J., *et al. Proceeds of the National Academy of Science, USA* 1996, **93**: 10212-10216.
6. Gale, A. J., *et al. Blood* 2000, **96**: 585-593.

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Definition of Symbols

	Consult instructions for use
	Manufacturer
	Refer to Safety Data Sheet
CONT	Contains…
	Temperature Limitation
LOT	Lot Number
	Expiration Date
REF	Catalog Number