

SPECTROZYME® TH

REF 238, 238L, 238B

Description

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

Formula: H-D-CHA-Ala-Arg-pNA.2AcOH
 (C₂₄H₃₈N₈O₅ · 2 C₂H₄O₂)

Molecular Weight: 638.7

Chemical Name: H-D-cyclohexylalanyl-alanyl-arginine-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, via RP-HPLC: $\geq 95\%$

Solubility: Up to 25 mg/mL in 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 8.0

Temperature: 37°C

Substrate: 0.5 mM REF 238

Enzyme: 5 nM human Thrombin (REF 470HT)

Kinetics: K_m : 3.26 μM K_{cat} : 272.5 sec^{-1}

Presentation

REF 238 amber glass vial containing 5 μmoles (3.19 mg) of lyophilized substrate.

REF 238L amber glass vial containing 50 μmoles (31.9 mg) of lyophilized substrate.

REF 238B amber glass vial containing 0.5 grams (ca. 764 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 concentration for SPECTROZYME TH is 1.0 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. Protect from moisture by allowing vial to reach room temperature prior to opening.

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. Do not submit to freeze-thaw cycles).

Warnings and Precautions

For Research Use Only.

CONT

H-D-cyclohexylalanyl-alanyl-arginine-para-nitroanilide diacetate salt



Warning

Hazard

Statements:

H315 Causes skin irritation.
 H319 Causes serious eye irritation
 H335 May cause respiratory irritation

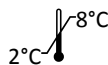
Precautionary Statements:

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.

References

1. Sonder, S. A., *et al.* *Clinical Chemistry* 1986, **32**: 934-937.
2. Brezniak, D. V., *et al.* *Biochemistry* 1990, **29**: 3536-3542.
3. Iyer, L., *et al.* *Thrombosis Research* 1995, **78**: 259-263.
4. Weiss, H. J., *et al.* *Blood* 1997, **89**: 1599-1611.
5. van 't Veer, C., *et al.* *Journal of Biological Chemistry* 1997, **272**: 4637-4377.
6. Hayes, K. L. and Tracey, P. B. *Journal of Biological Chemistry* 1999, **274**: 972-980.
7. Shriver, Z., *et al.* *Proc. Nat. Acad. Sci.* 2000, **97**: 10365-10370.
8. Safa, O., *et al.* *Journal of Biological Chemistry* 2001, **276**: 1829-1836.
9. Butenas, S., *et al.* *Blood* 2002, **99**: 923-930.

Definition of Symbols

	Consult instructions for use
	Manufacturer
	Refer to Safety Data Sheet
	Contains...
	Temperature Limitation: Store at 2°C to 8°C
	Lot Number
	Expiration Date
	Catalog Number