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BIOMEDICA DIAGNOSTICS

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SPECTROFLUOR™ FIXa

REF 299F and 299FL

Description

A fluorogenic substrate for the amidolytic assay of factor IXa activity in purified preparations.

Formula:	CH_3SO_2-D-CHG-Gly-Arg-AMC·AcOH
Molecular Weight:	665.8
Chemical Name:	methylsulfonyl-D-cyclohexylglycyl-glycyl-arginine-7-amino-4-methylcoumarin acetate salt
Composition:	Enzymatically digestible substrate colyophilized with glycine excipient
Purity:	≤ 0.5% free AMC
Solubility:	> 10 mM in distilled/deionized water > 1.0 mM in 0.05 M Tris Buffer, pH 7.4

Optical Characteristics:

Absorption Maximum Wavelength, λ_{Abs}: 342 nm
Emission Maximum Wavelength, λ_{Em}: 440 nm

Assay Conditions/Substrate Kinetics

Enzyme activity is determined by measuring the increase in fluorescence of the free fluorophore (AMC) generated, in comparison to the original substrate, per unit time at λ_{440} nm. At excess substrate concentration, the rate of fluorescence increase due to the amount of fluorophore 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 fluorometer (initial-rate-method).

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

Substrate:	25 µL of SPECTROFLUOR™ FIXa at a 10 mM stock concentration
Buffer:	200 µL of 50 mM Tris, 100 mM NaCl, 5 mM $CaCl_2$, 40% ethylene glycol, pH 7.4
Enzyme:	20 µL of human Factor IXa (REF 449B) at a 19.4 µg/mL concentration

Incubate for 3 minutes at 25°C (room temperature).

Kinetics: K_m: 0.23 mM V_{max}: 28.1 µmole/min

Presentation

REF 299F	Amber glass vial containing 10 µmoles of lyophilized substrate.
REF 299FL	Amber glass vial containing 50 µmoles of lyophilized substrate.

Reconstitution

REF 299F	Dissolve substrate with 1 mL of filtered deionized water to generate a 10 mM stock solution.
REF 299FL	Dissolve substrate with 5 mL of filter deionized water to generate 10 mM stock solution.

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 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

CONT	7-amino-4-methylcoumarin

For Reseach Use Only

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 229, SPECTROZYME® FIXa, *a chromogenic substrate*

REF 449B, human Factor IXa

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