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SPECTROFLUOR™ FXa

REF 222F

Description

A fluorogenic substrate for the amidolytic assay of factor Xa and for reactions in which factor Xa is generated.

Formula:	CH_3SO_2-D-CHA-Gly-Arg-AMC·AcOH
Molecular Weight:	679.8
Chemical Name:	methylsulfonyl-D-cyclohexylalanyl-gylcyl-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

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_{Em} 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).

Using the assay conditions listed, the following substrate kinetics were found:

Buffer: 50 mM Tris-HCl, 100 mM NaCl, 0.5% HSA, pH 7.4
Temperature: 25°C
Substrate: 1.5 mM (in water) REF 222F

1. Combine 900 µL of Buffer, 100 µL of Factor Xa (12 nM, REF 526) and 100 µL of REF 222F.
2. Incubate at 25°C and measure the fluorescence emission at 440 nm.

K_m: 220 µM k_{cat}: 120 sec^{-1}

Presentation

Amber glass vial containing 10 µmoles (6.8 mg) of lyophilized, digestible substrate.

Reconstitution

Reconstitute with 1 mL of filtered distilled/deionized water to create a stock solution with a concentration of 10.0 mM. Further dilutions may be made using a buffer of 50 mM Tris-HCl, 100 mM NaCl, 0.5% HSA, 7.4.

Warnings and Precautions

For Research Use Only.

CONT	7-amino-4-methylcoumarin



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.

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).

Related Products

REF 526, human Factor Xa

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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