

# 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<sub>3</sub>SO<sub>2</sub>-D-CHA-Gly-Arg-AMC·AcOH

Molecular Weight: 679.8

Chemical Name: methylsulfonyl-D-cyclohexylalanyl-glycyl-arginine-7-amino-4-methylcoumarin acetate salt

Composition: Enzymatically digestible substrate lyophilized with glycine excipient

Purity: ≤ 0.5% free AMC

Solubility: ≥ 10 mM in distilled/deionized water

### Optical Characteristics:

Absorption Maximum Wavelength, λ<sub>Abs</sub>: 342 nm

Emission Maximum Wavelength, λ<sub>Em</sub>: 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<sub>Em</sub> 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<sub>m</sub>: 220 μM      k<sub>cat</sub>: 120 sec<sup>-1</sup>

## 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
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### 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

## Definition of Symbols

	Consult instructions for use
	Manufacturer
	Refer to Safety Data Sheet
<b>CONT</b>	Contains...
	Temperature Limitation
<b>LOT</b>	Lot Number
	Expiration Date
<b>REF</b>	Catalog Number