



SPECTROFLUOR™ FIXa

REF 299F and 299FL

Description

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

Formula: CH₃SO₂-D-CHG-Gly-Arg-AMC•AcOH

Molecular Weight: 665.8

Chemical Name: methylsulfonyl-D-cyclohexylglycyl-

glycyl-arginine-7-amino-4methylcoumarin acetate salt

Composition: Enzymatically digestible substrate

colyophilized with glycine excipient

Purity: $\leq 0.5\%$ 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 CaCl₂,

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 μ mole/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 H315 Causes skin irritation.

Statements: H319 Causes serious eye irritation

H335 May cause respiratory irritation

Precautionary P261 Avoid breathing dust.

Statements: P264 Wash thoroughly after handling.

P280 Wear protective gloves/eye protection

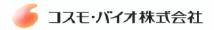
P337 + P313 If eye irritation persists: Get

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

