コスモ・バイオ株式会社

BIOMEDICA DIAGNOSTICS

BioMedica Diagnostics
500 West Avenue
Stamford, CT 06902 USA
1.902.798.5105
www.biomedicadiagnostics.com

SPECTROZYME® FXa

REF 222, 222L, 222B

Description

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

Formula:	CH_3O-CO-D-CHG-Gly-Arg-pNA.AcOH ($C_{24}H_{36}N_8O_7 \cdot C_2H_4O_2$)
Molecular Weight:	608.7
Chemical Name:	Methoxycarbonyl-D-cyclohexylglycyl-glycyl-arginine-para-nitroanilide acetate
Composition:	Enzymatically digestible substrate colyophilized with glycine
Extinction Coefficient:	$\varepsilon_{405\ nm}$ = 9650 $M^{-1} \cdot cm^{-1}$
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).

Using the following assay, factor X activity in plasma may be measured:

Buffer: 50 mM Tris, pH 8.4
Temperature: 37°C
Substrate: 5 mM (in water) REF 222
100 mM $CaCl_2$
20 µg/mL Russell's Viper Venom (RVV-X)

Prepare a 1:1 mix of Russell's Viper Venom and $CaCl_2$. Dilute samples and calibrators 1:20 with Tris buffer.

1. Add 50 µL of diluted standard or plasma sample to a well of a 96-well microtitre plate and incubate at 37°C for 4 minutes.
2. Add 50 µL of the RVV/$CaCl_2$ mixture.
3. Incubate at 37°C for 2 minutes.
4. Add 50 µL of 5 mM Spectrozyme FXa.
5. Incubate at 37°C for exactly 10 minutes.
6. Stop the reaction by adding 50 µL of 20% acetic acid and measure the absorbance at 405 nm using a microwell plate reader.

Presentation

REF 222	amber glass vial containing 5 µmoles (3.04 mg) of lyophilized, digestible substrate.
REF 222L	amber glass vial containing 50 µmoles (30.4 mg) of lyophilized, digestible substrate.
REF 222B	amber glass vial containing 0.5 grams of lyophilized substrate.

Reconstitution

Reconstitute with 1 mL (for REF 222) or dissolve in 10 mL (for REF 222L) of filtered distilled/deioniezed water to create a stock solution with a concentration of 5.0 mM. Dilute further to the working concentration with a buffer of 0.05 M Tris, pH 8.4. A typical working concentration for SPECTROZYME FXa is 0.5 - 2.5 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).

(continued)

Warnings and Precautions

For Reseach Use Only.

CONT	Methoxycarbonyl-D-cyclohexylglycyl-gylcyl-arginine-para-nitroanilide acetate

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. Dittmar, Sabine, *et al. Biochemical Journal* 1997, **321**: 787-793.
2. Rezaie, Alireza, R. *Journal of Biological Chemistry* 2000, **275**: 3320-3327.
3. Chuang, Y, *et al. Journal of Biological Chemistry* 2001, **276**: 14961-14971.
4. Edwards, Susan. T., *et al. Thrombosis Research* 2002, **106**: 71-79.
5. Dietzen, Dennis J., *et al. Thrombosis and Haemostasis* 2003, **89**: 65-73.
6. Kittur, F. S., *et al. Journal of Biological Chemistry* 2004, **279**: 24189-24196.

Definition of Symbols

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