



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: CH₃O-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: $\epsilon_{405 \text{ nm}} = 9650 \text{ M}^{-1} \cdot \text{cm}^{-1}$

Purity, via RP-HPLC: ≥ 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 CaCl₂

20 µg/mL Russell's Viper Venom (RVV-X)

Prepare a 1:1 mix of Russell's Viper Venom and CaCl₂. Dilute samples and calibrators 1:20 with Tris buffer.

 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/CaCl₂ mixture.
- 3. Incubate at 37°C for 2 minutes.
- 4. Add 50 μ L of 5 mM Spectrozyme FXa.
- 5. Incubate at 37°C for exactly 10 minutes.
- 6. Stop the reaction by adding 50 μ L 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

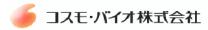
Reconstitute with 1 mL (for REF 222) or dissolve in 10 mL (for REF 222L) of filtered distilled/deioniezed water to create a stock solution with a concentration of 5.0 mM. Dilute further to the working concentration with a buffer of 0.05 M Tris, pH 8.4. A typical working concentration for SPECTROZYME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



Warning

Hazard

H315 Causes skin irritation.

Statements:

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:
- 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

