ENGLISH

S-2222™

For Laboratory Use Only

For General Laboratory Use

S-2222™

S-2222 is a chromogenic substrate for Factor Xa. It is also very sensitive to trypsin.

COMPOSITION

Each vial contains chromogenic substrate S-2222 25 mg and mannitol 120 mg as a bulking agent.

PRECAUTIONS AND WARNINGS:

Hazard class: None

Hazard statements: None

Precautionary statements: None

Supplemental Hazard Information: Up to 2.5% of the mixture consists of component of unknown acute toxicity (oral, dermal, inhalation) for the human health and unknown hazard to the aquatic environment.

CHEMISTRY

Chemical name:	N-Benzoyl-L-isoleucyl-L-glutamyl-glycyl-L-arginine-p-nitroaniline hydrochloride and its methyl ester
Formula:	◯-CO-Ile-Glu-(-OR)-Gly-Arg-pNA · HCl 50% where R is H and 50% where R is CH_3.
Mol. wt:	734.3 (R = H) and 748.3 (R = CH_3)
$\varepsilon_{316\ nm}$:	$1.27 \cdot 10^4\ mol^{-1} \cdot L \cdot cm^{-1}$
Solubility:	6 mmol/L in H_2O 2 mmol/L in Tris buffer (pH 8.3, I 0.25)
Stability:	Substance: Stable at 2-8°C for more than 3 years. The substance is somewhat hygroscopic and should be stored dry. Solution: 4 mmol/L in H_2O is stable for at least 6 months at 2 to 8°C. Contamination by microorganisms may cause hydrolysis.
Suitable stock solution:	1-4 mmol/L in H_2O. Vigorous shaking or an ultrasonic bath is recommended for dissolution, which is slow.

PRINCIPLE

$$\text{Bz-Ile-Glu-Gly-Arg-pNA} \xrightarrow{\text{Enzyme}} \text{Bz-Ile-Glu-Gly-Arg-OH+pNA}$$

The method for the determination of activity is based on the difference in (yellow) absorbance optical density between the pNA formed and the original substrate. The rate of pNA formation, i.e. the increase in absorbance per second at 405 nm, is proportional to the enzymatic activity and is conveniently determined with a photometer.

CHROMOGENIX

Instrumentation Laboratory Company - Bedford, MA 01730-2443 (USA)

Instrumentation Laboratory SpA-V.le Monza 338-20128 Milano (Italy)

Tel. +1 800 678 0710

301925R3

KINETIC DATA

Factor Xa (bovine): K_m=3 · 10-4 mol/L. k_{cat}=100 sec^{-1} in 37°C
Tris buffer pH 8.3, I 0.25

Trypsin (porcine): K_m=2 . 10-5 mol/L, k_{cat}=280 sec^{-1} in 37°C
Tris buffer pH 9.0, I 0.25

STANDARDIZATION

An activity of ΔA/min=0.05 (37°C) is obtained by using a substrate concentration of 2 · $k_m$and:

1. 0.1 nkat/mL of Factor Xa (Chromogenix) at pH 8.
2. Normal plasma diluted 1: 150 and activated with 6 µg RVV (Sigma) per mL of the dilution.

The same activity is obtained by using 5 · 10^{-13} mol/L of porcine trypsin (Novo). The substrate is also sensitive to subtilisin, acrosin and Factor XIIa but insensitive to most other enzymes tested, e.g. Factor IXa, kallikrein (glandular and plasma) and papain-like enzymes.

APPLICATIONS

The substrate has been used for the determination of:

1. FX in plasma (1,2)
2. FXa in plasma (3)
3. FXa inhibitor in plasma (4,5)
4. Heparin in plasma (6,7,8)
5. Factor VIII in plasma (9,10)
6. Coagulating enzyme from horseshoe crab
7. Trypsin in duodenal fluid (12)

1. AURELL L et al.: A new sensitive and highly specific chromogenic peptide substrate for Factor Xa. Thromb Res, 11, 595-609 (1977).
2. Chromogenix AB: Determination of Factor X in plasma. Laboratory Instruction.
3. VINAZZER H: Assay of Factor Xa with a chromogenic substrate. New methods for the analysis of coagulation using chromogenic substrates. I Wiff (Ed) de Gruyter, Berlin, 203-210 (1977).
4. ØDEGÅRD O R et al: Antifactor Xa activity measured with amidolytic methods. Haemostasis, 265-275 (1976).
5. Chromogenix AB: Determination of antifactor Xa in plasma. Laboratory Instruction.
6. TEIEN A N et al.: Assay of heparin in plasma using a chromogenic substrate for activated Factor X. Thromb Res, 8, 413-416 (1976).
7. TEIEN A N & LIE M: Evaluation of an amidolytic heparin assay method. Thromb Res, 10, 399-410 (1977).
8. Chromogenix AB: Determination of heparin in plasma. Laboratory Instruction.
9. ROSÉN S. Assay of factor VIII:C with a chromogenic substrate. Scand J Haematol, 33, Suppl 40: 139-45 (1984).
10. ROSÉN S. et al.: Clinical application of a chromogenic substrate method for determination of factor VIII activity. Thromb. Haemostasis, 54, 818-823 (1985).
11. SCULLY M F et al.: Evaluation of a chromogenic method for endotoxin measurement. Thromb Res., 20, 263-270 (1980).
12. Chromogenix AB: Determination of Trypsin in duodenal fluid. Laboratory Instruction.

CHROMOGENIX

S-2222

Printed Insert Sheet:	301925
Revision:	R3
Issued:	December 2016
C.O.:	473109

LANGUAGES

ENGLISH

TECHNICAL SPECS

PAPER:	White paper, 50-60 g/m^2 weight.
SIZE:	4.1 x 5.9" (104 x 150 mm.).
PRINT:	Front/Back.
PRINT COLOR:	All type in black.