

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.

CHEMISTRY

Chemical name: N-Benzoyl-L-isoleucyl-L-glutamyl-glycyl-L-arginine-p-nitroaniline hydrochloride and its methyl ester

Formula: $\text{O}-\text{CO}-\text{Ile}-\text{Glu}(-\text{OR})-\text{Gly}-\text{Arg}-\text{pNA} \cdot \text{HCl}$ 50% where R is H and 50% where R is CH_3 .

Mol. wt: 734.3 (R = H) and 748.3 (R = CH_3)

$\epsilon_{316 \text{ nm}}$: $1.27 \cdot 10^4 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$

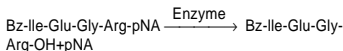
Solubility: 6 mmol/L in H_2O
2 mmol/L in Tris buffer (pH 8.3, I 0.25)

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

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

KINETIC DATA

Factor Xa (bovine): $K_m=3 \cdot 10^{-4}$ mol/L, $k_{cat}=100$ sec⁻¹
in 37°C

Tris buffer pH 8.3, I 0.25

Trypsin (porcine): $K_m=2 \cdot 10^{-5}$ mol/L, $k_{cat}=280$ sec⁻¹
in 37°C

Tris buffer pH 9.0, I 0.25

STANDARDIZATION

An activity of $\Delta A/\text{min}=0.05$ (37°C) is obtained by using a substrate concentration of $2 \cdot k_m$ 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 $5 \cdot 10^{-13}$ 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:

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



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