

# S-2222™

## CHROMOGENIX

Chromogenix-  
Instrumentation Laboratory SpA  
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301925R0

ENGLISH

### S-2222™

For Laboratory Use Only

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  $\text{CH}_3$ .

**Mol. wt:** 734.3 (R = H) and 748.3 (R =  $\text{CH}_3$ )

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

**Solubility:** 6 mmol/L in  $\text{H}_2\text{O}$   
2 mmol/L in Tris buffer (pH 8.3, I 0.25)

**Stability:** Substance: Stable at 25°C for more than 3 years. The substance is somewhat hygroscopic and should be stored dry.  
Solution: 4 mmol/L in  $\text{H}_2\text{O}$  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  $\text{H}_2\text{O}$ . Vigorous shaking or an ultrasonic bath is recommended for dissolution, which is slow.

#### PRINCIPLE

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

#### KINETIC DATA

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

**Trypsin (porcine):**  $K_m=2 \cdot 10^{-5} \text{ mol/L}$ ,  $k_{cat}=280 \text{ sec}^{-1}$  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  $k_m$  and:

- 0.1 nkat/mL of Factor Xa (Chromogenix) at pH 8.
- Normal plasma diluted 1:150 and activated with 6  $\mu\text{g}$  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:

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

DEUTSCH

### S-2222™

Nur für Laborzwecke

S-2222 ist ein chromogenes Substrat für Faktor Xa. Es ist außerdem sensitiv für Trypsin.

#### ZUSAMMENSETZUNG

Eine Flasche enthält 25 mg chromogenes Substrat S-2222, und 120 mg Mannitol als Füllstoff.

#### CHEMIE

**Chemischer Name:** N-Benzyl-L-Isoleucin-L-Glutaminsäure-Glycin-L-Arginin-p-Nitroanilin Hydrochlorid und seine Methylester

**Chem. Formel:**  $\text{O}-\text{CO}-\text{Ile}-\text{Glu}(-\text{OR})-\text{Gly}-\text{Arg}-\text{pNA} \cdot \text{HCl}$   
50% R = H; 50% R =  $\text{CH}_3$

**Molekulargewicht:** 734,3 (R = H) und 748,3 (R =  $\text{CH}_3$ )

**$\epsilon_{316 \text{ nm}}$ :**  $1,27 \cdot 10^4 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$

**Löslichkeit:** 6 mmol/l in  $\text{H}_2\text{O}$   
2 mmol/l in Tris Puffer (pH 8,3, I 0,25)



## REFERENCES

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***CHROMOGENIX***

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