

S-2366™

For Laboratory Use Only

For General Laboratory Use

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S-2366 is a chromogenic substrate for factor XI and activated protein C.

COMPOSITION

Each vial contains chromogenic substrate S-2366 25 mg and mannitol 40 mg as a bulking agent.

CHEMISTRY

Chemical name: L-Pyroglutamyl-L-prolyl-L-arginine-p-Nitroaniline hydrochloride.

Formula: < Glu-Pro-Arg-pNA · HCl

Mol. wt: 539.0

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

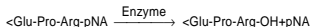
Solubility: > 10 mmol/L in H₂O

Stability: Substance: Stable until expiry date if stored at 2-8°C. Avoid exposure to light. The substance is hygroscopic and should be stored dry. Solution: 2 mmol/L in H₂O is stable for more than 6 months at 2 to 8°C.

Contamination by microorganisms may cause hydrolysis.

Suitable stock solution:

2-3 mmol/L in H₂O.

PRINCIPLE

The method for the determination of activity is based on the difference in 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

Protein C:

$K_m = 2 \cdot 10^{-4}$ mol/L and $k_{cat} = 80 \text{ sec}^{-1}$ (The enzyme is assumed to be pure. Mol. wt. 62 000) Determined with RVV activated *bovine* Protein C in 0.05 mol/L Tris, pH 8.0, I 0.25 (NaCl) and 4mmol/L CaCl_2 at 37°C.

$K_m = 8 \cdot 10^{-4}$ mol/L and $k_{cat} = 160 \text{ sec}^{-1}$.

Determined with thrombin-trombomodulin complex activated *human* Protein C in 0.05 mol/L Tris, pH 8.0, I 0.13 (NaCl) and 10 mmol/L CaCl_2 at 25°C (5).

FXI_a:

$K_m = 4 \cdot 10^{-4}$ mol/L and $k_{cat} = 1000 \text{ sec}^{-1}$ in 0.1 mol/L Phosphate buffer, pH 7.6, I 0.15 mol/L (NaCl) at 37°C (1).

$K_m = 5.6 \cdot 10^{-4}$ mol/L and $k_{cat} = 350 \text{ sec}^{-1}$ in 0.09 mol/L Tris, pH 8.3, 0.09 mol/L NaCl, 1 mg/mL of bovine serum albumin at room temperature.(4).

SELECTIVITY

S-2366 is also readily split by trypsin, thrombin, plasmin and tissue plasminogen activator (2). It is split by FXII_a, plasma kallikrein and FX₃ as well.

APPLICATIONS

The substrate can be used for the determination of purified enzyme preparations as well as of Protein C and FXI in plasma (1,3,4,5)



1. SCOTT C F et al.: Amidolytic assay of human factor XI in plasma. Comparison with a coagulant assay and a new rapid radioimmunoassay. *Blood* 63, 42-50 (1984).
2. FRIBERGER P et al.: Activity of plasminogen activators on tripeptide chromogenic substrates. In *Progress in Chemical Fibrinolysis and Thrombolysis Vol 4*. Davidson J F et al Churchill Livingstone 149-153 (1979).
3. BERTINA R M et al.: The use of a function and immunologic assay for plasma Protein C in the study of the heterogeneity of congenita Protein C deficiency. *Thromb Haem* 51, 1-5 (1984).
4. VAN DER GRAAF et al.: Isolation and functional characterisation of the active light chain of activated human blood coagulation Factor XI. *J Biol Chem* 258, 9669-9675 (1983).
5. SALA N et al.: A functional assay of Protein C in human plasma. *Blood* 63, 671-675 (1984).

CHROMOGENIX