ENGLISH

S-2238™

For Laboratory Use Only

For General Laboratory Use

S-2238™

S-2238 is a chromogenic substrate for thrombin.

COMPOSITION

Each vial contains chromogenic substrate S-2238 25 mg and mannitol 120 mg as a bulking agent.

CHEMISTRY

Chemical name:	H-D-Phenylalanyl-L-pipecolyl-L-arginine-p-nitroaniline dihydrochloride.
Formula:	H-D-Phe-Pip-Arg-pNA · 2 HCl
Mol. wt.:	625.6
$\varepsilon_{316\ nm}$:	$1.27 \cdot 10^4\ mol^{-1} \cdot L \cdot cm^{-1}$
Solubility:	> 10 mmol/L in H_2O
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: 1 mmol/L in H_2O is stable for more than 6 months at 2-8°C. Contamination by microorganisms may cause hydrolysis.
Suitable stock solution:	1-2 mmol/L in H_2O.

PRINCIPLE

$$\text{H-D-Phe-Pip-Arg-pNA} \xrightarrow{\text{Enzyme}} \text{H-D-Phe-Pip-Arg-OH+pNA}$$

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

Instrumentation Laboratory Company - Lexington, MA 02421-3125 (USA)

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

301929R1

KINETIC DATA

Human thrombin: $K_m = 0.7 \cdot 10^{-5}$ mol/L
$V = 1.7 \cdot 10^{-7}$ mol/min · NIH-U

Bovine thrombin: $K_m = 0.9 \cdot 10^{-5}$ mol/L
$V = 2.2 \cdot 10^{-7}$ mol/min · NIH-U

Both determined at 37°C in 2.5 mL 0.05 mol/L Tris buffer pH 8.3, I 0.15.

STANDARDIZATION

An activity of ΔA/min = 0.05 (37°C) is obtained by using 0.1 mmol/L substrate and:

1. 0.03 NIH-U/mL of bovine thrombin (Roche or Parke-Davis)
2. 0.04 U/mL of human thrombin (MRC standard thrombin)

Aprotinin (Trasylol®) may be added to the buffer in a concentration of 75 KIU/L in order to inhibit other activities than that of thrombin.

Note: For thrombin standardization against the MRC-standard the natural substrate fibrinogen, is recommended as the primary substrate. The clotting and amidolytic activities of degraded thrombins do not always develop in parallel.

APPLICATIONS

The substrate has been used for the determination of:

1. Prothrombin in plasma (1,2)
2. Antithrombin in plasma (3,4)
3. Platelet factor 3 in plasma (5,6)
4. Heparin in plasma (7)

1. AXELSSON G et al.: Prothrombin determination by means of chromogenic peptide Substrate. Thromb Haemost 36, 517 (1976).
2. BERGSTRÖM K & EGBERG N: Determination of plasma prothrombin using a synthetic chromogenic substrate. Paper read at the 2nd European Congress on Clinical Chemisty, October 1976.
3. ABILGAARD et al.: Antithrombin (heparin cofactor) assay with "new" chromogenic substrates. Thromb Res II, 549-553 (1977).
4. Chromogenix AB: Determination of antithrombin-heparin cofactor in plasma. Laboratory Instruction.
5. SANDBERG H & ANDERSSON L O: A highly sensitive assay of platelet factor 3 using a chromogenic substrate. Thromb Res 14, 113-124 (1979).
6. SANDBERG H et al.: Determination of platelet factor 3 in whole blood by a chromogenic peptide substrate assay. Thromb Res 18, 871-882 (1980).
7. Chromogenix AB: Determination of heparin in plasma. Laboratory Instruction.

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