

S-2288™

For Laboratory Use Only

For General Laboratory Use

S-2288™

S-2288 is a chromogenic substrate sensitive to a broad spectrum of serine proteases.

COMPOSITION

Each vial contains chromogenic substrate S-2288, 25 mg and mannitol 40 mg as a bulking agent.

CHEMISTRY

Chemical name: H-D-Isoleucyl-L-prolyl-L-arginine-p-nitroaniline dihydrochloride.

Formula: H-D-Ile-Pro-Arg-pNA · 2HCl

Mol. wt: 577.6

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

Solubility: > 40 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 in a dry place.

Solution: 10 mmol/L in H₂O is stable for more than two months at 2-8°C. Contamination by micro-organisms may cause hydrolysis.

Suitable

stock solution: 10 mmol/L in H₂O

KINETIC DATA

The following kinetic constants were estimated at 37°C in Tris buffer pH 8.4, I 0.15.

Enzyme	K_m (mol/L)	V_{max} (mol/min and unit)	K_{cat} (sec ⁻¹)
Thrombin	$3 \cdot 10^{-6}$	$10 \cdot 10^{-8}$	118
Urokinase	$2 \cdot 10^{-4}$	$2 \cdot 10^{-10}$	16
Factor XII f	$4 \cdot 10^{-4}$	$5 \cdot 10^{-5}$	23
t-PA ¹⁾ one-chain	$1 \cdot 10^{-3}$	$10 \cdot 10^{-11}$	26
t-PA ¹⁾ two-chain	$3 \cdot 10^{-4}$	$11 \cdot 10^{-11}$	28
Plasma kallikrein	$1 \cdot 10^{-3}$	$13 \cdot 10^{-7}$	-
Plasmin	$9 \cdot 10^{-3}$	$5 \cdot 10^{-6}$	181
Factor Xa	$2 \cdot 10^{-3}$	$9 \cdot 10^{-8}$	110
C_{is}	$3 \cdot 10^{-3}$	$3 \cdot 10^{-6}$	4
C_{tr}	$6 \cdot 10^{-4}$	$1 \cdot 10^{-6}$	2

¹⁾ Tissue plasminogen activator (porcine)

CHROMOGENIX

Units expressed in nmols are as follows:

Thrombin (human) Ortho	1 NIH U	= $1.4 \cdot 10^{-2}$ nmol
Urokinase Leo	1 Ploug U	= $1.8 \cdot 10^{-4}$ nmol
Factor XII ^a Mol wt 28 000	1 mg	= 36 nmol
t-PA (one- and two-chain) Mol wt 64 000	1 IU	= $6.3 \cdot 10^{-5}$ nmol(1)
Plasma kallikrein	U	= μ mol/min
Plasmin (human)Chromogenix 1 CU		≈ 0.45 nmol
Factor Xa (bovine) Diagen	1 U (Denson)	= $1.4 \cdot 10^{-2}$ nmol
C _{is} ^a and C _{ir} ^a Mol wt 85 000	1 mg	= 12 nmol

*The enzyme is assumed to be pure

STANDARDIZATION

With a substrate concentration of $1 \cdot 10^{-3}$ mol/L and an enzyme concentration of $4 \cdot 10^{-9}$ mol/L the following activities are obtained.

Enzyme	$\Delta A/\text{min}$	Enzyme	$\Delta A/\text{min}$	Enzyme	$\Delta A/\text{min}$
Thrombin	0.275	Plasmin	0.042	Factor Xa	0.084
Urokinase	0.031	t-PA (one-chain)	0.030	C _{is}	0.002
Factor XII ^a	0.023	t-PA (two-chain)	0.040	C _{ir}	0.003

The tissue plasminogen activator is not affected by the following inhibitors used at the concentrations given.

1. Trasylol 30 KIU/mL.
2. Soybean trypsin inhibitor 50 μ g/mL.
3. Antithrombin 0.1 PEU/mL and heparin 3 IU/mL.

APPLICATIONS

The substrate has been used for the determination of Tissue plasminogen activator in purified preparations (2,3,4)



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2. WALLÉN P RÅNBY M: Peptides of the Biological Fluids. Brussels, Belgium. Abstr. 67, (1980)
3. FRIBERGER P et al: Progr. in Chem. Fibr. and Thromb. IV Ed JF Davidson. 149 (1979)
4. FRIBERGER P: Thesis Scand J clin lab Invest 42 suppl 162, 58-59 (1982)
5. Chromogenix AB: Determination of Tissue Plasminogen Activator (t-PA) in purified preparations. Laboratory instructions.
6. Chromogenix AB: Determination of proteolytic activity in plasma, serum or Euglobulin fractions. Laboratory instructions.

CHROMOGENIX