

**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 H<sub>2</sub>O

*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 H<sub>2</sub>O 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 H<sub>2</sub>O

**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 <sup>-1</sup> )
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 <sup>1)</sup> one-chain	$1 \cdot 10^{-3}$	$10 \cdot 10^{-11}$	26
t-PA <sup>1)</sup> 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
$C_{ir}$	$6 \cdot 10^{-4}$	$1 \cdot 10^{-6}$	2

<sup>1)</sup> 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 <sup>a</sup> 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	= $\mu$ mol/min
<b>Plasmin (human) Chromogenix</b>	<b>1 CU</b>	= <b>0.45 nmol</b>
Factor Xa (bovine) Diagen	1 U (Denson)	= $1.4 \cdot 10^{-2}$ nmol
C <sub>is</sub> <sup>a</sup> and C <sub>ir</sub> <sup>a</sup> 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 <sub>is</sub>	0.002
Factor XII <sup>a</sup>	0.023	t-PA (two-chain)	0.040	C <sub>ir</sub>	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  $\mu$ 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)



1. WALLÉN P RÅNBY M: Personal communication.
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**

**S-2288**

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**LANGUAGES**

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ENGLISH

**TECHNICAL SPEC'S**

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PAPER: White paper,  
50-60 g/m<sup>2</sup> weight.  
SIZE: 4.1 x 5.9" (104 x 150 mm.).  
PRINT: Front/Back.  
PRINT COLOR: All type in black.