

S-2586™

For Laboratory Use Only

For General Laboratory Use

S-2586™**CHROMOGENIX****COMPOSITION**

Each vial contains chromogenic substrate S-2586 25 mg and mannitol 40 mg as a bulking agent.

CHEMISTRY

Chemical name: 3-Carbomethoxypropionyl-L-arginyl-L-prolyl-L-tyrosine-p-nitroaniline hydrochloride

Formula: MeO-Suc-Arg-Pro-Tyr-pNA · HCl

Mol. wt.: 705.3

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

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: 1 mmol/l in H₂O is stable for more than one month at 2 to 8°C. Contamination by micro-organisms may cause hydrolysis.

Suitable stock solution:

1 mmol/l in H₂O

PRINCIPLE

Enzyme

MeO-Suc-Arg-Pro-Tyr-pNA → MeO-Suc-Arg-Pro-Tyr-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.

KINETIC DATA

Chymotrypsin:

$K_m = 5 \cdot 10^{-5} \text{ mol/l}$ and

$K_{cat} = 140 \text{ sec}^{-1}$.

(Mol.wt. 25000. The enzyme is assumed to be pure.)

Determined in 0.03 mol/l Tris pH 8.3, I 0.4 with 3 mmol/l CaCl₂ and at 37°C.

STANDARDIZATION

An activity of $\Delta A/\text{min} = 0.05$ (37°C) is obtained by using a substrate concentration of $2 \cdot K_m$ and 0.03 mg/l of α -Chymotrypsin Worthington ($65 \mu \text{ BTEE/mg}$) and similar activities were obtained by using enzymes from three other sources (Merck, Boehringer and Sigma).

SELECTIVITY

The substrate is not split by trypsin, thrombin, factor Xa, plasma or glandular kallikreins, plasmin or urokinase at concentrations that readily split ($\Delta A/\text{min} \approx 1$) commercial-Arg-pNA substrates. Granulocyte elastase has very low activity on the substrate. Cathepsin G is known to split this type of substrates, but the activity per mg of enzyme is in the order of one per cent of that of chymotrypsin. Pancreas elastase free from chymotrypsin impurities does not split the substrate.

APPLICATIONS

The substrate has hitherto been used for the assay of purified preparations (1) and for the assay of antichymotrypsin in blood plasma (2). It has also been used for the assay of chymotrypsin-like activity in other biological samples (3). It may also be used for the assay of chymotrypsin in pancreatic juice.



REFERENCES

1. Chromogenix: Standardization of Chymotrypsin. Laboratory instruction.
2. BERGSTRÖM K: Personal communication.
3. BERDAL B P et al: Demonstration of extracellular chymotrypsin-like activity from various Legionella species. J Clin Microbiol, 1982, 16, 452-457.

CHROMOGENIX
