

PEPSTATIN A

From Microbial Source

Product Number **P 4265** Storage Temperature: 2-8 °C

CAS #: 26305-03-3

Product Description

Appearance: White powder Molecular formula: $C_{34}H_{63}N_5O_9$ Molecular weight: 685.9

Structure: 1 Isovalery-Val-Val-Sta-Ala-Sta

where Sta = statine = (3S,4S)-4-amino-3-

hydroxy-6-methylheptanoic acid K_i for Pepsin:² approximately 10⁻¹⁰ M.

Pepstatin A is an inhibitor of acid proteases (aspartyl peptidases). It forms a 1:1 complex with proteases such as pepsin, ^{1,2} renin, ^{1,2} cathepsin D, ^{1,2} bovine chymosin, ² and protease B (*Aspergillus niger*). The inhibitor is highly selective ⁴ and does not inhibit thiol proteases, neutral proteases or serine proteases. Solublized γ-secretase ⁵ and retroviral protease ⁶ are also inhibited by Pepstatin A. It has been used to characterize proteases from several sources. Pepstatin A is thought to inhibit by a collected-substrate inhibition mechanism.

This inhibitor is often used as a component in a final mixture with other inhibitors (as in Sigma Protease Inhibitor Cocktails). One recommended set of stock solution concentrations is: bestatin (1.7 mM, selective for aminopeptidase), E-64 (0.22 mM, for cysteine proteases), Pepstatin A (2.5 mM, for aspartyl proteases), AEBSF (18 mM, for serine proteases) and disodium EDTA (86 mM, for metalloproteases). 10

Preparation Instructions

Pepstatin A is only sparingly soluble in water. ⁴ It is normally dissolved in a solvent such as ethanol, methanol, or DMSO and diluted into buffer. The stock solution should be of a concentration that the solvent used is diluted at least 1000X in the working solution.

It has been dissolved at 10 mg/mL in ethanol with heat. The resulting solution is colorless, but may appear

ProductInformation

hazy. To remove haziness, add up to 50 μl of glacial acetic acid per mL of ethanol.

At 25 mg/mL DMSO Pepstatin A forms a clear, faint yellow solution.

Stock solutions at 1 mg/mL should be stable at least a week at 4 °C. A 1 mM solution in methanol or DMSO should be stable for months at -20 °C. If solutions become more yellow the reagent is hydrolyzing.

An effective working concentration is 1 μ M, stable for at least one day at room temperature. ¹⁰ A typical working concentration is 0.5-1.0 μ g/mL.

Storage/Stability

When stored at 2-8 $^{\circ}\text{C}$ this product has a shelf life of 3 years.

References

- Umezawa, H., Methods in Enzymology, 45, 689 (1976).
- 2. Marciniszyn, J., et al., *Adv. Exp. Med. Biol.* **95**, 199 (1977).
- 3. Takahashi, K., and Chang, W. J., *J. Biochem.* (*Tokyo*), **80**, 497 (1976).
- 4. Dunn, B.M., in *Proteolytic Enzymes: A Practical Approach*, R.J. Beynon and J.S. Bond, eds. (IRL Press, 1989), p. 63.
- 5. Li, Y.-M., et al., *Proc. Natl. Acad. Sci USA*, **97**, 6138 (2000).
- 6. Katoh, I., et al., Nature, 329, 654 (1987).
- 7. Arima, K., et al., *Phytochemistry*, **54**, 559 (2000).
- 8. Farias, M. E., and Manca de Nadra, M. C., *FEM Microbiol. Lett.* **185**, 263 (2000).
- 9. Rich, D. H., et al. *Biochemistry*, **24**, 3165 (1985).
- Proteolytic Enzymes: A Practical Approach, R.J. Beynon and J.S. Bond, eds. (IRL Press, 1989), p. 245 (Appendix III).
- 11. Umezawa, J., et al., J. Antibiotics, 23, 259 (1970).
- 12. Morishima, H. et al., *J. Antibiotics*, **25**, 551 (1972).

 JWM 3/16/01