

## Product Information

### PEPSTATIN A

from microbial source

Product Number **P 5318**

Storage Temperature 2-8 °C

CAS #: 26305-03-3

#### Product Description

Appearance: White powder

Molecular formula: C<sub>34</sub>H<sub>63</sub>N<sub>5</sub>O<sub>9</sub>

Molecular weight: 685.9

Structure:<sup>1</sup> Isovalery-Val-Val-Sta-Ala-Sta

where Sta = statine = (3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid

K<sub>i</sub> for Pepsin:<sup>2</sup> approximately 10<sup>-10</sup> M.

Pepstatin A is an inhibitor of acid proteases (aspartyl peptidases). It forms a 1:1 complex with proteases such as pepsin,<sup>1,2</sup> renin,<sup>1,2</sup> cathepsin D,<sup>1,2</sup> bovine chymosin,<sup>2</sup> and protease B (*Aspergillus niger*).<sup>3</sup> The inhibitor is highly selective<sup>4</sup> and does not inhibit thiol proteases, neutral proteases or serine proteases. Solubilized  $\gamma$ -secretase<sup>5</sup> and retroviral protease<sup>6</sup> are also inhibited by Pepstatin A. It has been used to characterize proteases from several sources.<sup>7,8</sup> Pepstatin A is thought to inhibit by a collected-substrate inhibition mechanism.<sup>9</sup>

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).<sup>10</sup>

#### Preparation Instructions

Pepstatin A is only sparingly soluble in water.<sup>4</sup>

The solubility of Pepstatin A is related to the purity of the preparation. P 5318, a purer form (minimum 90%) of Pepstatin A than P 4265, is insoluble at any concentration in methanol or DMSO. It can be dissolved at 1 mg/mL in 10% (v/v) acetic acid in methanol (9:1 methanol:acetic acid). The inclusion of acetic acid is necessary to dissolve this peptide in methanol or DMSO. It has been dissolved at

1-2 mg/ml in ethanol, but heat may be required for complete solution. Solutions of Pepstatin A can be heated as high as 60 °C without any decomposition of the peptide. The recommended procedure is to include acetic acid.

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

An effective working concentration is 1  $\mu$ M, stable for at least one day at room temperature.<sup>10</sup> A typical working concentration is 0.5-1.0  $\mu$ g/mL.

#### Storage/Stability

When stored at 2-8 °C this product has a shelf life of three years.

#### References

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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).
10. *Proteolytic Enzymes: A Practical Approach*, R.J. Beynon and J.S. Bond, eds. (IRL Press, 1989), p. 245 (Appendix III).

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