

Product Information

Leupeptin

BioUltra, hemisulfate salt, microbial, ≥ 95% (HPLC)

L5793

Product Description

CAS Number: 103476-89-7

Synonyms: Acetyl-Leu-Leu-Arg-al,
N-Acetyl-L-leucyl-L-leucyl-L-argininal hemisulfate salt

Molecular Weight: 475.6 (anhydrous)

Molecular Formula: C₂₀H₃₈N₆O₄ • ½ H₂SO₄

Leupeptin is a reversible competitive inhibitor of cysteine proteases and serine proteases.¹ Leupeptin acts by covalent binding to, respectively:²

- Catalytic cysteines of cysteine proteases
- Catalytic series of serine proteases

Leupeptin was first isolated from microbial sources as a mixture of two very similar forms:³

- Acetyl-Leu-Leu-Arg-al
- Propionyl-Leu-Leu-Arg-al

While the propionyl leupeptin is active as an inhibitor, the acetyl form is more commonly used.

Leupeptin has been reported to inhibit calpain,⁴ cathepsin B,⁵ cathepsins H and L,⁶ and trypsin.⁷ A typical working concentration range is 10-100 µM. The activity of leupeptins and related analogs has been studied.⁹ Table 1 lists inhibitory activities of leupeptin against various enzymes.¹⁰

HPLC analysis of leupeptin gives multiple peaks because of the formation of tautomeric isomers in solution.¹¹ The primary mechanism of inactivation of leupeptin is via racemization of the L-arginal moiety, as leupeptin with a D-arginal group is totally inactive.¹⁰ If the aldehyde is oxidized but retains its L-configuration, the resulting carboxylate compound does have some inhibitory activity.¹²

Leupeptin hemisulfate was the first commercially available leupeptin salt form. This product is a chemically synthetic form of leupeptin hemisulfate. Several publications¹³⁻¹⁵ and dissertations¹⁶ have cited use of product L5793 in their protocols.

Table 1. Concentrations for 50% inhibition (IC₅₀, reported as µg/mL leupeptin)¹⁰

Enzyme	Substrate	IC ₅₀ (µg/mL)
Aspergillopepsin II (Proctase A)	Casein	> 250
Aspergillopepsin I (Proctase B)	Casein	> 250
Cathepsin A	Carbobenzoxy-L-glutamyl-L-tyrosine (Cb-Glut-Tyr)	1680
Cathepsin B	N ^α -benzoyl-L-arginine amide HCl	0.44
Cathepsin D	Hemoglobin	109
α-Chymotrypsin	Casein	> 500
β-, γ-, and δ-Chymotrypsin	Casein	> 500
Kallikrein	BAEE (N ^α -benzoyl-L-arginine ethyl ester HCl)	75
Papain	Casein	0.5
Pepsin	Casein Hemoglobin	> 500 > 500
Plasmin	Fibrinogen	8
Thrombin	N ^α -(p-toluene-sulfonyl)-L-arginine methyl ester HCl	10000
Thrombokinas	Plasma	15
Trypsin	Casein	2

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the lyophilized product at -20 °C.

Solubility

This product is tested for solubility in water at 50 mg/mL.

A 10 mM aqueous solution of leupeptin has been reported to be stable for a week at 4 °C, and for a month at -20 °C.⁸ At working concentrations (10-100 µM), a solution is stable for only a few hours.⁸ The stock solution should be stored on ice for intermittent use over several hours.

Usage

Because of its aldehyde group, leupeptin may act as a reducing agent, and thus may interfere in protein determination assays, such as the Lowry assay and, to a lesser extent, the Bradford assay.

References

1. Umezawa, H., *Methods Enzymol.*, **45**, 678-695 (1976).
2. Kędzior, M. *et al.*, *Med. Microbiol. Immunol.*, **205(4)**, 275-279 (2016).
3. Aoyagi, T. *et al.*, *J. Antibiot. (Tokyo)*, **22(6)**, 283-286 (1969).
4. Zimmerman, U.-J.P. and Schlaepfer, W.W., *Biochemistry*, **21(17)**, 3977-3983 (1982).
5. Knight, C.G., *Biochem. J.*, **189(3)**, 447-453 (1980).
6. Zollner, H., *Handbook of Enzyme Inhibitors*, 2nd ed., Part B. VCH Press, pp. 821-822 (1993).
7. Kuramochi, H. *et al.*, *J. Biochem.*, **86(5)**, 1403-1410 (1979).
8. Beynon, R., and Bond, J.S., *Proteolytic Enzymes: A Practical Approach* (2nd ed.). Oxford University Press (Oxford, UK), p. 323 (2001).
9. Maeda, K. *et al.*, *J. Antibiotics*, **24(6)**, 402-404 (1971).

10. Umezawa, H., *Pure Appl. Chem.*, **33(1)**, 129-144 (1973).
11. Saino, T. *et al.*, *Chem. Pharm. Bull.*, **30(7)**, 2319-2325 (1982).
12. Billinger, E. *et al.*, *FEBS Open Bio.*, **10(12)**, 2605-2615 (2020).
13. Ganassi, M. *et al.*, *Mol. Cell*, **63(5)**, 796-810 (2016).
14. Buchwalter, A. *et al.*, *eLife*, **8**, e49796 (2019).
15. Fu, L. *et al.*, *mBio*, **12(5)**, e02220-e02221 (2021).
16. Hangel, Christoph, "Leptin-mediated downregulation of glutathione peroxidase 4 and iron overload contributing to podocyte ferroptosis in diabetic nephropathy". Ruperto Carola University of Heidelberg, Dr. rer. nat. dissertation, pp. 33, 36 (2019).

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