

Product Information

Leupeptin

Trifluoroacetate salt, microbial, ≥ 90% (HPLC)

L2023

Product Description

CAS Number: 147385-61-3

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

Molecular Weight: 426.55 (free base basis)

Molecular Formula: C₂₀H₃₈N₆O₄ • x C₂HF₃O₂

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

Several theses¹³ and dissertations¹⁴⁻¹⁶ have cited use of product L2023 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	<i>N</i> ^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 (<i>N</i> α-benzoyl-L-arginine ethyl ester HCl)	75
Papain	Casein	0.5
Pepsin	Casein	> 500
	Hemoglobin	> 500
Plasmin	Fibrinogen	8
Thrombin	<i>N</i> α-(<i>p</i> -toluene-sulfonyl)-L-arginine methyl ester HCl	10000
Thrombokinase	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

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