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ProductInformation

LEUPEPTIN
Prod. Nos. L 2023, L 9783,
L 0649, L 2884 and L 8511

SYNONYM: Acetyl-Leu-Leu-Arg-al

Prod. No.	Salt form	Source	CAS Number	Molecular formula	Mol. Wt.	Purity (HPLC)
L2023	Trifluroacetate	microbial	147385-61-3	$C_{20}H_{38}N_6O_4$	426.6	≥90%
L9783	Hydrochloride	microbial	24125-16-4	$C_{20}H_{38}N_6O_4 \cdot HCl$	463.0	≥90%
L0649	Hydrochloride	microbial	24125-16-4	$C_{20}H_{38}N_6O_4 \cdot HCl$	463.0	≥70%
L2884	Hemisulfate	microbial	103476-89-7	$C_{20}H_{38}N_6O_4 \cdot ½H_2SO_4$	475.6	≥90%
L8511	Hemisulfate	synthetic	103476-89-7	$C_{20}H_{38}N_6O_4 \cdot ½H_2SO_4$	475.6	≥85%

PHYSICAL DESCRIPTION:

Appearance: White powder, sometimes with faint yellow cast
Molecular formula: Each includes salt for all but L 2023
Formula weight: Does not include water content
Structure: Acetyl-leucyl-leucyl-arginal (arginal=arginine-aldehyde) Only the L-arginal is active.

STORAGE / STABILITY AS SUPPLIED:

If the powder is stored frozen and kept very dry, it should be stable at least two years.

SOLUBILITY / SOLUTION STABILITY:

Salts of leupeptin are reported to be soluble in water, ethanol, acetic acid and DMF. Sigma's assays show that all but L 8511 give a clear solution at 50 mg/mL in water (L 8511 may give a slightly hazy solution.) A 10 mM aqueous solution is stable for a week at 4 °C, and at least 6 months as frozen aliquots at –20 °C. At working concentrations (10 to 100 μM) a solution is stable only a few hours; the stock solution should be stored on ice for intermitted use over several hours.[1]

The primary mechanism of inactivation is racemization of the L-arginal; the D-arginal form is totally inactive. If the aldehyde is oxidized but retains its L-configuration, the resulting compound does have some inhibitory activity.[2]

As noted in product specification, HPLC analysis of leupeptin will show multiple peaks due to tautomeric isomers that form in solution.[3]

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GENERAL REMARKS:

Leupeptin is a reversible competitive inhibitor of serine and thiol proteases.[4] It has been reported to inhibit calpain[5], cathepsin B[6], cathepsins H and L[7] and trypsin[8]. A typical working concentrations is in the range of 10 to 100 μM.[7]

Leupeptin appears to be equally effective in any salt form, adjusting for equivalent peptide content. The hemisulfate salts were the first to be commercially available. Of the three salts, the hydrochloride is the least invasive form in biological settings. No problem or preference for the trifluoroacetate (TFA) form has been notes; TFA is volatile, so could possibly be removed by lyophilization.[9]

Microbially produced leupeptin inhibitor was first isolated as a mixture of two very similar forms: acetyl-Leu-Leu-Arg-al and propionyl-Leu-Leu-Arg-al.[10] Although the propionyl leupeptin is active as an inhibitor (Prod. No. L 3402), the acetyl form is more commonly used and is available from Sigma in several different forms, as described above.

Reported concentrations for 50% inhibition (as μg/mL leupeptin)[11]

Plasmin	8	
Trypsin	2 (casein as substrate)	K_i = 0.13 μM (BAEE as Substrate)
Papain	0.5	
Cathepsin B	0.44	K_i approx. 7 nanomolar
Pepsin	no inhibition	
Cathepsins A,D	no inhibition	
Thrombin	no inhibition	
alpha-Chymotrypsin	no inhibition	

Leupeptin, because of its aldehyde group, may act as a reducing agent and therefore interfere in protein determinations such as Lowry and, to a lesser extent, Bradford.[12] The activity of leupeptins and related analogs has been studied.[13]

GENERAL REMARKS: (continued)

For broad inhibition of proteases, several protease inhibitors are commonly used for convenience in a "cocktail" to be added to a lysis buffer. Sigma offers several prepared cocktail mixtures, two of which contain leupeptin. Many variations have been published, but one suggested formulations is given below:

Leupeptin	(1 µM final concentration, for serine and thiol proteases);
PMSF	(200 µM final concentration, for serine proteases);
pepstatin	(1 µM final concentration, for acidic proteases);
EDTA	(100 µM final concentration, for metalloproteses)[7]

REFERENCES:

1. *Proteolytic Enzymes: A Practical Approach*, Beynon, R.J. and J.S. Bond, eds. (IRL Press, 1989).
2. Sigma production.
3. Saino, T. et al., *Chem. Pharm. Bull.*, 30 (7), 2319 (1982).
4. Umezawa, H., *Methods in Enzymology*, 45, 679-683 (1976).
5. Zimmerman, U-J. P. and W.W. Schlaepfer, *Biochemistry*, 21, 3977 (1982).
6. Knight, C.G., *Biochem. J.*, 189, 447 (1980).
7. Zollner, H., *Handbook of Enzyme Inhibitors*, 2nd. ed. Part B (VCH Press, 1993), pp 821-22.
8. Kuramochi, H. et al., *J. Biochem.*, 86, 1403 (1979).
9. Sigma production.
10. Aoyagi, T., *J. Antibiotics*, 22, 283 (1969).
11. Sigma files or production.
12. Maeda, K. et al., *J. Antibiotics*, 24 (6), 402 (1971).