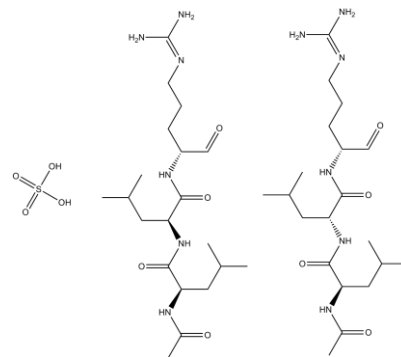


## Product Data Sheet

### Chemical Properties

<b>Product Name:</b>	Leupeptin, Microbial
<b>Cas No.:</b>	103476-89-7
<b>M.Wt:</b>	493.6
<b>Formula:</b>	C <sub>20</sub> H <sub>38</sub> N <sub>6</sub> O <sub>4</sub> ·1/2H <sub>2</sub> SO <sub>4</sub>
<b>Synonyms:</b>	Leupeptin hemisulfate salt microbial, L-Leucinamide, Leupeptin, Microbial



<b>Chemical Name:</b>	2-acetamido-N-(1-((5-((diaminomethylene)amino)-1-oxopentan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)-4-methylpentanamide
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<b>Canonical SMILES:</b>	<chem>O=C(C(C([H]))([H])C(C([H]))([H])[H])([H])C([H])([H])[H])([H])N([H])C(C([H]))([H])[H]=O)N([H])C(C(N([H])C(C([H])=O)([H])C([H])([H])C([H])([H])C([H])([H])/N=C(N([H])[H])/N([H])[H]=O)([H])C([H])([H])C(C([H])([H])[H])([H])C([H])([H])[H]</chem>
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<b>Solubility:</b>	≥24.7mg/mL in DMSO
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<b>Storage:</b>	Store at -20°C The product is not stable in solution, please dissolve it immediately before use.
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<b>General tips:</b>	For obtaining a higher solubility, please warm the tube at 37° C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20° C for several months.
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<b>Shopping Condition:</b>	Evaluation sample solution : ship with blue ice All other available size: ship with RT, or blue ice upon request
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### Biological Activity

<b>Targets :</b>	Proteases
<b>Pathways:</b>	Serine Protease
<b>Description:</b>	

Leupeptin is a reversible inhibitor of protease with  $K_i$  values of 35 nM, 3.4  $\mu$ M, 6 nM and 72 nM for bovine trypsin, human plasmin, bovine spleen cathepsin B and recombinant human calpain, respectively [1, 2].

As a protease inhibitor, leupeptin was originally isolated from the *Streptomyces* species. It exerted poor membrane permeability due to its polar C-terminal. For calpain, leupeptin showed moderate potent activities with  $IC_{50}$  values of 0.211  $\mu$ M, 1.8  $\mu$ M and 0.938  $\mu$ M against the enzymes isolated from porcine erythrocyte, porcine kidney and human platelet, respectively. In cultured MRC-C cells, leupeptin suppressed the growth of human coronavirus strain 229E through inhibited the activity of trypsin. The mean  $IC_{50}$  value was 0.8  $\mu$ M [2, 3].

#### **Reference:**

1. Mehdi S. Cell-penetrating inhibitors of calpain. *Trends in biochemical sciences*, 1991, 16: 150-153.
2. Krauser J A, Powers J C. 6.1 BIOLOGICAL ROLES. *Proteinase and Peptidase Inhibition: Recent Potential Targets for Drug Development*, 2003: 144.
3. Appleyard G, Tisdale M. Inhibition of the growth of human coronavirus 229E by leupeptin. *Journal of general virology*, 1985, 66(2): 363-366.

## **Protocol**

### **Cell experiment:**

Cell lines	MRC-C cells infected with HCV 229E
Preparation method	The solubility of this compound in DMSO is $\geq 49.35$ mg/mL. General tips for obtaining a higher concentration: Please warm the tube at 37°C for 10 minutes and/or shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.
Reacting conditions	0, 1, 10 and 100 $\mu$ g/mL; 24 hrs
Applications	In cultures of MRC-C cells, Leupeptin prevented multiplication of the human coronavirus strain 229E. The $IC_{50}$ value of Leupeptin in plaque tests was 0.4 $\mu$ g/mL, whilst growth of host cells was unaffected by Leupeptin at 50 $\mu$ g/mL. In single-cycle growth experiments, Leupeptin (100 $\mu$ g/mL) reduced virus yield only if added within 2 hrs of infection, indicating its action on an early stage of virus replication.

### **Animal experiment [3]:**

Animal models	C57BL/6NCrl male mice
Dosage form	0, 9, 18 36 and 40 mg/kg; i.p.

## Applications

Leupeptin was well tolerated by the animals and dose-dependently produced a substantial increase in LC3b-II in both the total tissue extracts and the lysosome enriched fraction (LE fraction). At the electron microscopy (EM) level, leupeptin induced the accumulation of electron-dense vesicular structures that, in hepatocytes, were visible by 60 min after treatment (40 mg/kg). The results suggested that Leupeptin enhanced LC3b-II levels in vivo by protecting this protein from being degraded inside lysosomes, and thus the leupeptin-based assay could be potentially used for studying the dynamics of macroautophagy in mice.

## Other notes

Please test the solubility of all compounds indoor, and the actual solubility may slightly differ with the theoretical value. This is caused by an experimental system error and it is normal.

## Reference:

[1]. Appleyard G, Tisdale M. Inhibition of the growth of human coronavirus 229E by leupeptin. *Journal of general virology*, 1985, 66(2): 363-366.

[2]. Haspel J, Shaik RS, Ifedigbo E, Nakahira K, Dolinay T, Englert JA, Choi AM. Characterization of macroautophagic flux in vivo using a leupeptin-based assay. *Autophagy*. 2011 Jun;7(6):629-42.

## Caution

**FOR RESEARCH PURPOSES ONLY.**

**NOT FOR HUMAN, VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.**

*Specific storage and handling information for each product is indicated on the product datasheet. Most ApexBio products are stable under the recommended conditions. Products are sometimes shipped at a temperature that differs from the recommended storage temperature. Shortterm storage of many products are stable in the short-term at temperatures that differ from that required for long-term storage. We ensure that the product is shipped under conditions that will maintain the quality of the reagents. Upon receipt of the product, follow the storage recommendations on the product data sheet.*

**ApexBio Technology**

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