

## Product Information

# Leupeptin

Hydrochloride salt, microbial,  $\geq 70\%$  (HPLC)**L0649**

## Product Description

CAS Number: 24125-16-4

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

Molecular Weight: 463.01 (anhydrous)

Molecular Formula:  $C_{20}H_{38}N_6O_4 \bullet HCl$ 

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

- 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:<sup>3</sup>

- 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,<sup>4</sup> cathepsin B,<sup>5</sup> cathepsins H and L,<sup>6</sup> and trypsin.<sup>7</sup> A typical working concentration range is 10-100  $\mu M$ . The activity of leupeptins and related analogs has been studied.<sup>9</sup> Table 1 lists inhibitory activities of leupeptin against various enzymes.<sup>10</sup>

HPLC analysis of leupeptin gives multiple peaks because of the formation of tautomeric isomers in solution.<sup>11</sup> 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.<sup>10</sup> If the aldehyde is oxidized but retains its L-configuration, the resulting carboxylate compound does have some inhibitory activity.<sup>12</sup>

Several dissertations<sup>13-15</sup> have cited use of product L0649 in their protocols.

**Table 1.** Concentrations for 50% inhibition ( $IC_{50}$ , reported as  $\mu g/mL$  leupeptin)<sup>10</sup>

| Enzyme  | Substrate  | $IC_{50}$<br>( $\mu g/mL$ ) |
|---|--|-----------------------------|
| 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> <sup>α</sup> -benzoyl-L-arginine amide HCl                              | 0.44                        |
| Cathepsin D                                       | Hemoglobin   | 109                         |
| $\alpha$ -Chymotrypsin                            | Casein   | > 500                       |
| $\beta$ -, $\gamma$ -, and $\delta$ -Chymotrypsin | Casein   | > 500                       |
| Kallikrein  | BAEE ( <i>N</i> <sup>α</sup> -benzoyl-L-arginine ethyl ester HCl)                | 75                          |
| Papain  | Casein   | 0.5                         |
| Pepsin  | Casein   | > 500                       |
|   | Hemoglobin   | > 500                       |
| Plasmin   | Fibrinogen   | 8                           |
| Thrombin  | <i>N</i> <sup>α</sup> -( <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  $-20\text{ }^{\circ}\text{C}$ .

## Solubility

This product is tested for solubility in water at 2 mg/mL. One dissertation cites preparation of stock solutions of this product in cold sterile water at 5 mg/mL, with storage in aliquots at  $-20\text{ }^{\circ}\text{C}$ .<sup>13</sup>

A 10 mM aqueous solution of leupeptin has been reported to be stable for a week at  $4\text{ }^{\circ}\text{C}$ , and for a month at  $-20\text{ }^{\circ}\text{C}$ .<sup>8</sup> At working concentrations (10-100  $\mu\text{M}$ ), a solution is stable for only a few hours.<sup>8</sup> 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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L0649pis Rev 07/22 CKV,GCY,MAM

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