Leupeptin

Catalog Number: EI002
Lot Number: TDD01

Specifications and Use

Product	♦ N-acetyl-Arg-Arg-aldehyde-HCl
Molecular Mass	♦ 463.0 Da
Purity	♦ > 95% by high performance liquid chromatography
Quantity	♦ 25 mg
Effective Concentration	♦ 10 - 100 μM
Activity and Applications	♦ Measured by its ability to inhibit rhCathepsin-L (R&D Systems, Catalog # 952-CY). ♦ The IC_{50} is < 2 nM, as measured under the described conditions. See Activity Assay Protocol on next page for details. ♦ Leupeptin is a reversible inhibitor of cysteine and trypsin-like serine proteases such as Cathepsin-L, papain, plasmin, and trypsin (1).
Formulation	♦ Supplied as a 50 mM solution in DMSO.
Dilution	♦ It is recommended that the first dilution be no less than 50 fold into an aqueous solution.
Storage	♦ Stable for 6 months after time of receipt when stored at -20° C to -80° C **in a manual defrost freezer.**

Reference:

1. Beynon, R. and J.S. Bond (2001) *Proteolytic Enzymes: A Practical Approach*, Oxford University Press.

R&D Systems, Inc.
1-800-343-7475

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, 5 mM DTT, pH 6.0
- Leupeptin (R&D Systems, Catalog # EI002), 50 mM stock in DMSO
- Recombinant human Cathepsin L (R&D Systems, Catalog # 952-CY)
- Substrate: Z-Leu-Arg-AMC (R&D Systems, Catalog # ES008), 2 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhCathepsin L to 40 µg/mL in Assay Buffer.
2. Incubate on ice for 15 minutes.
3. After incubation, dilute activated rhCathepsin L to 0.2 µg/mL in Assay Buffer.
4. Prepare a curve of Leupeptin in Assay Buffer. Make the following serial dilutions: 256 nM, 64 nM, 32 nM, 16 nM, 8 nM, 4 nM, 2 nM, and 0.5 nM.
5. Gently mix 30 µL of each of the Leupeptin curve dilutions with 30 µL of the 0.2 µg/mL rhCathepsin L. Include a control (in duplicate) containing 30 µL of Assay Buffer and 30 µL of 0.2 µg/mL rhCathepsin L.
6. Incubate mixtures at room temperature for 15 minutes.
7. Dilute reaction mixture by adding 90 µL of Assay Buffer to each reaction.
8. Dilute Substrate to 20 µM in Assay Buffer.
9. Load 50 µL of the incubated mixtures into a plate and start the reaction by adding 50 µL of 20 µM Substrate.
10. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
11. Derive the 50% inhibiting concentration (IC_{50}) for Leupeptin by plotting RFU/min (or specific activity) versus concentration with 4-PL fitting.
12. The specific activity of rhCathepsin L at each point may be determined using the following formula (if needed):

$$\text{Specific Activity (pmoles/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor**(pmole/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Derived using calibration standard 7-amino, 4-Methyl Coumarin (Sigma, Catalog # A-9891)

Final Assay Conditions Per Well

rhCathepsin L (MW: 25,524): 0.002 µg (0.784 nM)
Leupeptin curve: 25.6 nM, 6.4 nM, 3.2 nM, 1.6 nM, 0.8 nM, 0.4 nM, 0.2 nM, and 0.05 nM
Substrate: 10 µM