Pepstatin A

Catalog Number: EI003
Lot Number: TDG01

Specifications and Use

Product	♦ Isovaleryl-Val-Val-Statine-Ala-Statine ♦ Statine = (3S, 4S)-4-amino-3-hydroxy-6-methylheptanoic acid
Molecular Mass	♦ 685.91 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 rmCathepsin D (R&D Systems, Catalog # 1029-AS). ♦ The IC_{50} is < 4 nM, as measured under the described conditions. See Activity Assay Protocol for details. ♦ Pepstatin A is a reversible inhibitor of aspartic proteases such as pepsin, renin, and Cathepsin D (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.**

References:

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: 0.1 M NaOAc, 0.2 M NaCl, pH 3.5
- Pepstatin (R&D Systems, Catalog # EI003)
- rmCathepsin D (R&D Systems, Catalog # 1029-AS)
- Substrate: MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH_2 (R&D Systems, Catalog # ES001), 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 rmCathepsin D to 20 µg/mL in Assay Buffer.
2. Incubate for 10 minutes at room temperature.
3. Prepare a curve of Pepstatin A in Assay Buffer. Make the following serial dilutions: 20000 nM, 500 nM, 50 nM, 25 nM, 15 nM, 7.5 nM, 3.75 nM, and 0.375 nM.
4. Dilute rmCathepsin D to 2 µg/mL in Assay Buffer.
5. Combine 30 µL of each of the Pepstatin A curve dilutions with 30 µL of 2 µg/mL rmCathepsin D. Include a rmCathepsin D control in duplicate containing 30 µL of Assay Buffer and 30 µL of 2 µg/mL rmCathepsin D without Pepstatin A.
6. Incubate mixtures at room temperature for 15 minutes.
7. Dilute reaction mixtures by adding 90 µL Assay Buffer to each.
8. Dilute Substrate to 20 µM in Assay Buffer.
9. Load into a black well plate 50 µL of the incubated mixtures and start the reaction by adding 50 µL of 20 µM Substrate.
10. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively in kinetic mode for 5 minutes.
11. Derive the 50% inhibiting concentration (IC_{50}) for Pepstatin A by plotting RFU/min (or specific activity) versus concentration with 4-PL fitting.
12. The specific activity for rmCathepsin D 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 MCA-Pro-Leu-OH (Bachem, Catalog # M-1975)

Final Assay Conditions Per Well

rmCathepsin D (MW: 44,283): 0.02 µg (4.51 nM)
Pepstatin A curve: 2000 nM, 50 nM, 5 nM, 2.5 nM, 1.5 nM, 0.75 nM, 0.375 nM, 0.038 nM, and 0 nM
Substrate: 10 µM