# *Bestatin*

**Catalog Number:** EI005
**Lot Number:** TDC01

## *Specifications and Use*

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|---|---|
| **Product** | ♦ (2S,3R)-3-amino-2-hydroxy-4-phenylbutanoyl-Leu-HCl |
| **Molecular Mass** | ♦ 344.8 Da |
| **Purity** | ♦ > 95% by high performance liquid chromatography |
| **Quantity** | ♦ 10 mg |
| **Effective Concentration** | ♦ 50 - 500 μM |
| **Activity and Applications** | ♦ Measured by its ability to inhibit recombinant human Aminopeptidase N (R&D Systems, Catalog # 3815-ZN).<br>♦ The $IC_{50}$ is < 2 μM as measured under the described conditions. See Activity Assay Protocol on next page for details.<br>♦ Bestatin is a reversible inhibitor of aminopeptidases and leukotriene A4 hydrolase (1). |
| **Formulation** | ♦ Supplied as a 50 mM solution in DMSO. |
| **Dilution** | ♦ It is recommended that the first dilution be no less than 25 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: 50 mM MES, pH 7.0
- Bestatin (R&D Systems, Catalog # EI005), 50 mM stock in DMSO
- Recombinant human Aminopeptidase N (APN) (R&D Systems, Catalog # 3815-ZN)
- Substrate: Ala-AMC (Bachem, Catalog # I-1410), 10 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. Prepare a curve of Bestatin. Make serial dilutions in Assay Buffer to the following concentrations: 2000 µM, 1000 µM, 200 µM, 40 µM, 8 µM, 2 µM, 1 µM, 0.1 µM, and 0.01 µM.
2. Dilute rhAPN to 2 µg/mL in Assay Buffer.
3. Combine 30 µL of each dilution of the Bestatin curve with 30 µL of 2 µg/mL rhAPN. Include an APN blank, in duplicate, containing 30 µL rhAPN and 30 µL of Assay Buffer without any Bestatin.
4. Incubate reaction mixtures for 15 minutes at room temperature.
5. Dilute each reaction mixture by adding 90 µL Assay Buffer.
6. Dilute Substrate to 200 µM in Assay Buffer.
7. Load 50 µL of each dilute reaction mixture into a plate, and start the reaction by adding 50 µL of 200 µM Substrate.
8. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively in kinetic mode for 5 minutes.
9. Derive the 50% inhibition concentration ($IC_{50}$) of Bestatin by plotting RFU/min (or specific activity) versus concentration with 4-PL fitting.
10. The specific activity of rhAPN at each point can 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 # A9891).

**Final Assay Conditions Per Well**

rhAPN (MW: 104,003): 0.020 µg (0.00192 µM)
Bestatin: 200 µM, 100 µM, 20 µM, 4 µM, 0.8 µM, 0.2 µM, 0.1 µM, 0.01 µM, and 0.001 µM
Substrate: 100 µM