



Phosphoramidon

Catalog Number: EI006

Lot Number: TDE02

Specifications and Use

Product	◆ N-(α -Rhamnopyranosyloxyhydroxyphosphinyl)-L-leucyl-L-tryptophan (Phosphoramidon)
Molecular Mass	◆ 587.5 Da
Purity	◆ > 95% by high performance liquid chromatography
Quantity	◆ 5 mg
Effective Concentration	◆ 1 - 10 μ M
Activity and Applications	<ul style="list-style-type: none">◆ Measured by its ability to inhibit Thermolysin (R&D Systems, Catalog # 3097-ZN).◆ The IC_{50} is < 2.0 nM, as measured under the described conditions. See Activity Assay Protocol on next page for details.◆ Phosphoramidon is a reversible inhibitor of thermolysin-like metalloproteases such as neprilysin and endothelin converting enzyme (1). It is most effective in mildly acidic conditions.
Formulation	◆ The disodium salt is dissolved in methanol at a concentration of 20 mM.
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.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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

Activity Assay Protocol

Materials

- ◆ Assay Buffer: 50 mM MES, 10 mM CaCl₂, pH 6.0
- ◆ Phosphoramidon (R&D Systems, Catalog # EI006)
- ◆ Thermolysin (R&D Systems, Catalog # 3097-ZN)
- ◆ Substrate: MCA-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(DNP)-OH (R&D Systems, Catalog # ES005), 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 Thermolysin to 0.2 µg/mL in Assay Buffer.
2. Prepare a curve of Phosphoramidon in Assay Buffer. Make the following serial dilutions: 400000, 10000, 200, 25, 10, 5, 2, 0.2 and 0.02 nM.
3. Combine 30 µL of each of the Phosphoramidon curve dilutions with 30 µL of 0.2 µg/mL Thermolysin. Include a Thermolysin control in duplicate containing 30 µL of Assay Buffer and 30 µL of 0.2 µg/mL Thermolysin without Phosphoramidon.
4. Incubate mixtures at room temperature for 15 minutes.
5. Dilute reaction mixtures by adding 90 µL of Assay Buffer to each.
6. Dilute Substrate to 20 µM in Assay Buffer.
7. Load into a black well plate 50 µL of the incubated mixtures, and start the reaction by adding 50 µL of 20 µM Substrate.
8. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
9. Derive the 50% inhibition concentration (IC₅₀) for Phosphoramidon by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
10. The specific activity for Thermolysin at each point may be determined using the following formula (if needed):

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

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975)

Final Assay Conditions Per Well

Thermolysin: 0.002 µg

Phosphoramidon curve: 40000 nM, 1000 nM, 20 nM, 2.5 nM, 1 nM, 0.5 nM, 0.2 nM, 0.02 nM, and 0.002 nM

Substrate: 10 µM