

Product Data Sheet

Product Name: Pyr-Arg-Thr-Lys-Arg-AMC

Cat. No.: GA23423

Chemical Properties

Cas. No. 155575-02-3

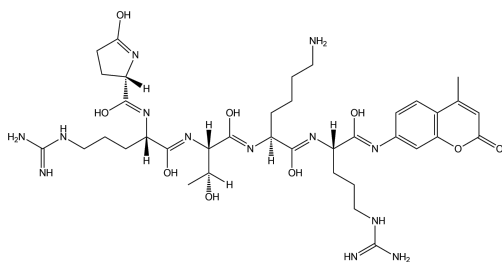
Formula $C_{37}H_{57}N_{13}O_9$ M.Wt 827.94

Solubility Soluble in DMSO Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Furin activity assay [1-3]

This plan is based on literature, please modify it to meet your specific needs.

1. Preparation of substrate working solution

(1) Prepare storage solution: Dissolve Pyr-Arg-Thr-Lys-Arg-AMC in DMSO.

Note: Unused storage solution should be aliquoted and stored in the dark at -20°C or -80°C to avoid repeated freezing and thawing.

(2) Prepare working solution: Dilute the storage solution with a reaction buffer (100mM HEPES buffer (pH=7.0) containing 0.2% Triton X-100, 2mM $CaCl_2$, 0.02% sodium azide and 1mg/ml BSA).

Note: Prepare the working solution fresh and store briefly at 4°C or on ice.

Caution: Product has not been fully validated for medical applications. For research use only.

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2. Preparation of furin enzyme working solution: Dilute furin enzyme with reaction buffer.

3. Prepare a 96-well plate.

Blank wells: Add Pyr-Arg-Thr-Lys-Arg-AMC substrate at a concentration of 10-20 μ M.

Reaction wells: Add a mixture of Pyr-Arg-Thr-Lys-Arg-AMC substrate and furin enzyme at concentrations of 10-20 μ M and 10-20nM, respectively.

Inhibitor control wells (optional, for verifying specificity): A mixture of furin enzyme and inhibitor.

4. Pre-incubate the 96-well plate at 37°C for 30min.

5. Measure the fluorescence intensity at 440-460nm under 350-360nm excitation using a fluorescence spectrophotometer.

Precautions:

1) Fluorescent substrate has quenching problems. Please avoid light as much as possible to slow down fluorescence quenching.

2) For your safety and health, please wear a lab coat and disposable gloves.

References:

[1] Braun E, Hotter D, Koepke L, et al. Guanylate-Binding Proteins 2 and 5 Exert Broad Antiviral Activity by Inhibiting Furin-Mediated Processing of Viral Envelope Proteins. *Cell Rep.* 2019;27(7):2092-2104.e10.

[2] Shiryayev SA, Farhy C, Pinto A, et al. Characterization of the Zika virus two-component NS2B-NS3 protease and structure-assisted identification of allosteric small-molecule antagonists. *Antiviral Res.* 2017;143:218-229.

[3] Ma XR, Alugubelli YR, Ma Y, et al. MPI8 is Potent against SARS-CoV-2 by Inhibiting Dually and Selectively the SARS-CoV-2 Main Protease and the Host Cathepsin L. *ChemMedChem.* 2022;17(1):e202100456.

Background

Pyr-Arg-Thr-Lys-Arg-AMC also called pERTKR-AMC, is a pentapeptide fluorescent substrate

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containing AMC (7-amino-4-methylcoumarin). Pyr-Arg-Thr-Lys-Arg-AMC can be efficiently cleaved by furin protease, and is therefore often used as a substrate for measuring furinase activity [1].

References:

[1] Neugebauer WA, Parent A, Yuan XW, Day R. C-terminal amidation on aryl hydrazine resin. *Adv Exp Med Biol.* 2009;611:371-372.

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