

Product Data Sheet

Product Name: Suc-AAPF-pNA

Cat. No.: GC44960

Chemical Properties

Cas. No. 70967-97-4

SMILES OC(CCC(N[C@@H](C)C(N[C@@H](C)C(N1CCC[C@H]1C(N[C@@H](CC2=CC=CC=C2)C(NC3=CC=C([N+](O-)=O)C=C3)=O)=O)=O)=O)=O

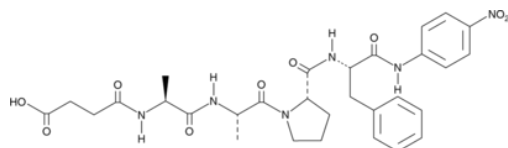
Formula C₃₀H₃₆N₆O₉ M.Wt 624.7

Solubility DMF: 5 mg/ml, DMSO: 5 mg/ml, DMSO: PBS (pH 7.2) (1:1): 0.5 mg/ml
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

This protocol only provides a guideline, and should be modified according to your specific needs.

1. Solution preparation

(1) Stock solution: Dissolve Suc-AAPF-pNA in DMSO to a final concentration of 10 mM.

Note: After unused storage solution is aliquoted, store Suc-AAPF-pNA at -20°C in the dark to avoid repeated freezing and thawing.

(2) Working solution: Dilute the stock solution with experimental buffer to the required working concentration, for example: 0.2 mM.

Note: Please adjust the optimal working concentration according to the actual situation or refer to the literature to set the gradient concentration by yourself. The working solution must be prepared and used immediately.

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

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2. Sensitivity Determination of Suc-AAPF-pNA to Protease Cleavage^[1] (from the literature, for reference only)

(1) Materials Preparation: Substrate: Suc-AAPF-pNA, Active enzyme, PBS buffer, 96-well microtiter plate, Microplate reader

(2) Reaction system: Add the chromogenic substrate Suc-AAPF-pNA (5 µl, final concentration 0.2 mM) and active enzyme (a few microliters, the amount depends on the enzyme activity) to each well, and add PBS to make the final volume reach 200 µl.

(3) Reaction conditions: Perform the reaction at a specific temperature, such as 20°C.

(4) Measure the absorbance spectrophotometrically at 405 nm using a microplate reader. Measure at the following time points: 0, 20, 40, 60, 120, 180, 240, 300, and 360 minutes. Control: Set up a blank well for each substrate without adding any enzyme.

(5) Data Collection and Analysis: Conduct reactions in triplicate. Subtract the blank measurement at each time point. Use the mean absorbance of the three reactions for plotting and analysis.

Notes:

This protocol provides guidance for determining sensitivity to protease cleavage using Suc-AAPF-pNA. It can be adapted based on other literature and specific experimental requirements.

References:

[1] Fu Z, Akula S, Qiao C, et al. Duodenases are a small subfamily of ruminant intestinal serine proteases that have undergone a remarkable diversification in cleavage specificity[J]. PLoS One, 2021, 16(5): e0252624.

Background

Suc-AAPF-pNA (Suc-Ala-Ala-Pro-Phe-pNA) is a colorimetrically specific substrate for elastase^[1]. Under the action of enzymes, Suc-AAPF-pNA is hydrolyzed, releasing yellow p-nitroaniline (pNA), and the change in absorbance at 400-410 nm is measured to reflect enzyme activity through colorimetry^[2]. Suc-AAPF-pNA also serves as a substrate for cathepsin G, bacillus protease, chymotrypsin, chymotrypsin, cyclophilin, and peptidyl-prolyl isomerase^[3].

The structure and group characteristics of Suc-AAPF-pNA are as follows:

(1) Succinyl group (Suc): Increases the water solubility and stability of the substrate.

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(2)Amino acid sequence (Ala-Ala-Pro-Phe): This specific sequence mimics certain natural substrates and helps study the specificity of proteases towards different substrates.

(3)p-Nitroaniline (pNA): A common chromophoric group that releases a yellow product with absorbance characteristics upon hydrolysis, facilitating the monitoring of enzyme reactions through spectrometry.

References:

- [1] Vinci V A. Biochemical and genetic analysis of serine proteases of *Streptomyces* spp[M]. The Ohio State University, 1988.
- [2] Chaves-Pozo E, Valero Y, Lozano M T, et al. Fish granzyme A shows a greater role than granzyme B in fish innate cell-mediated cytotoxicity, *Front. Immunol.* 10 (2019) 2579[J]. 2019.
- [3] Attucci S, Korkmaz B, Juliano L, et al. Measurement of free and membrane-bound cathepsin G in human neutrophils using new sensitive fluorogenic substrates[J]. *Biochemical Journal*, 2002, 366(3): 965-970.

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