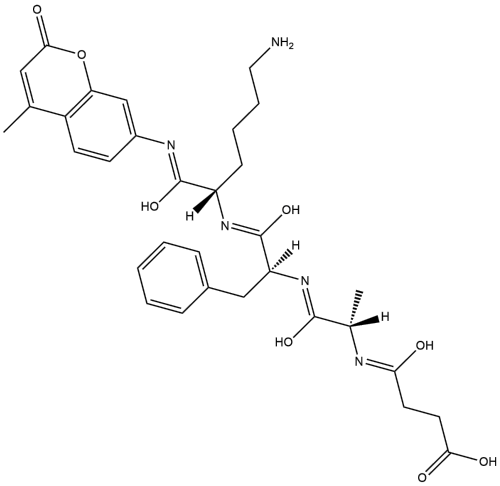
Product Data Sheet

Product Name: Suc-Ala-Phe-Lys-AMC
Cat. No.: GA23548

Chemical Properties

Cas. No.	73207-91-7		
Formula	$C_{32}H_{39}N_5O_8$	M.Wt	621.69
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

Determination of Plasmin Activity Using Suc-Ala-Phe-Lys-AMC[1]

This protocol is adapted from research data and provided for reference only. Adjustments may be necessary based on specific experimental requirements.

1. Reagent Preparation: Dissolve Suc-Ala-Phe-Lys-AMC in a buffer to prepare a working concentration substrate solution (e.g., 0.045mM Ala-Phe-Lys-AMC or 0.8mM Suc-Ala-Phe-Lys-AMC). Use 0.05M Tes/NaOH buffer (pH=7.5). Prepare a 20% (v/v) dimethyl sulfoxide solution as an auxiliary solvent; this concentration has no effect on enzyme activity. Prepare a series of plasmin

standard solutions with known activity (CTA units).

2. Sample Pretreatment: For crude extract samples, to avoid non-specific hydrolysis of the substrate by aminopeptidases, a protected substrate such as Suc-Ala-Phe-Lys-AMC must be used. Cell culture supernatant samples can be used directly after removing cell debris by centrifugation. When testing samples that may contain plasminogen activators (e.g., urokinase) or thrombin, the inhibitor Trasylol should be prepared.

3. Plasmin Activity Assay: Under conditions of 24°C, add the substrate solution, Tes buffer, and 20% DMSO to a final reaction volume of 0.5mL. Then, initiate the reaction by adding the enzyme solution (e.g., plasmin, urokinase, or thrombin) or the test sample. The reaction time is typically 5 minutes or can be adjusted based on enzyme activity.

4. Fluorescence Measurement and Quantification: Plasmin hydrolyzes Suc-Ala-Phe-Lys-AMC, releasing the fluorescent group 7-amino-4-methylcoumarin. After the reaction, measure the fluorescence intensity under standardized conditions (calibrate the fluorometer daily with 10nM 7-amino-4-methylcoumarin) using a spectrofluorometer. Calculate the enzyme activity by comparing the rate of fluorescence intensity change against a standard curve.

Precautions:

(1) This method is extremely sensitive, capable of detecting as little as 10^{-5}CTA units of plasmin. The reaction rate is linear over at least a 100-fold range of enzyme concentrations. By utilizing the inhibitor Trasylol and differences in the kinetic constants of the substrates, plasmin activity can be specifically assayed even in the presence of enzymes like thrombin or urokinase.

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

References:
[1] Pierzchala PA, Dorn CP, Zimmerman M. A new fluorogenic substrate for plasmin. Biochem J. 1979 Dec 1;183(3):555-9. doi: 10.1042/bj1830555.

Background

Suc-Ala-Phe-Lys-AMC is a highly sensitive fluorogenic substrate. Suc-Ala-Phe-Lys-AMC can be specifically recognized and cleaved by plasmin, releasing the AMC fluorophore. Enzyme activity is quantified by measuring the change in fluorescence intensity. Suc-Ala-Phe-Lys-AMC can be used for

Product Data Sheet

the detection and related research of plasmin and gingipain K[1-2].

References:

[1] Götz MG, James KE, Hansell E, et al. A new class of potent and selective inhibitors of asparaginyl endopeptidases (legumains) from evolutionarily diverse pathogens. J Med Chem. 2008 May 8;51(9):2816-32.

Caution: Product has not been fully validated for medical applications. For research use only.
Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA