Product Data Sheet

Product Name: Suc-Gly-Pro-Leu-Gly-Pro-AMC
Cat. No.: GA23560

Chemical Properties

Cas. No.	72698-36-3		
Formula	$C_{34}H_{44}N_6O_{10}$	M.Wt	696.76
Solubility	Soluble in DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ 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	Suc-Gly-Pro-Leu-Gly-Pro-AMC		

Protocol

Experiment[1]:

Sample	Human plasma
Preparation Method	In each reaction with a total volume of 100 µl of solution, 20 µl of human plasma was added to assay buffer consisting of 20 mM Tris/HCl, pH 8.0, 0.1 M NaCl and 1 mM EDTA. The substrate（Suc-Gly-Pro-Leu-Gly-Pro-AMC） was added to a final concentration of 20 µM. The samples were incubated at 37 °C, and fluorescence was recorded using a microtiter-plate fluorometer with an excitation wavelength of 360 nm and an emission wavelength at 460 nm.

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Reaction Conditions	20 μM Suc-Gly-Pro-Leu-Gly-Pro-AMC
Applications	The FAP activity in human plasma was measured using succinyl-pentapeptide composed of SUc-Gly-pro-Leu-Gly-pro-AMC, which contains two FAP cleavage sites.Only the cleavage between the last proline residue and the AMC group releases the fluorescent AMC group, resulting in a fluorescence reading, and the FAP activity of the measured samples ranges from 1.3 to 7 nM/min/ μL.

Cell experiment [2]:

Cell lines	Clone MC3T3-E1 cells
Preparation Method	Samples of 5 x 104 cells were cultured in 35-mm plastic dishes for 5 days. After the cultures were washed with 0.02 M Ca^{+2}- , Mg^{+2}-free phosphate buffer (pH 7.2), they were scraped off from the dishes and homogenized in the same buffer with a glass teflon homogenizer. The homogenate was used for the assay of Suc-Gly-Pro-Leu-Gly-Pro-AMC ase and DAP activities.
Reaction Conditions	30 μl of 2 mM Suc-Gly-Pro-Leu-Gly-Pro-AMC in 37°C for 90 min

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Applications	When examining the activities of collagenase-like peptidase (Suc-Gly-Pro-Leu-Gly-Pro-AMC ase) and dipeptidyl aminopeptidase (DAP) in Mc3t3-e1 in newly cloned osteoblasts with osteogenic ability, the Suc-Gly-Pro-Leu-Gly-Pro-AMC ase in MC3T3-El presented in this paper inhibited by heavy metals (Zn^{+2} and Cu^{+2}), and some reagents (Mn^{+2}, Ca^{+2}, Mg^{+2}, and EDTA) in creased the enzyme activity; especially, MnanEDTA increased the Suc-Gly-Pro-Leu-Gly-Pro-AMC ase activity to 8.2-fold and 23-fold[3].

References:

[1]. Zhen EY, Jin Z, et,al. Circulating FGF21 proteolytic processing mediated by fibroblast activation protein. Biochem J. 2016 Mar 1;473(5):605-14. doi: 10.1042/BJ20151085. Epub 2015 Dec 3. PMID: 26635356; PMCID: PMC4764976.

[2]. Chikuma, T., Ishii, Y., Kato, T. et al. Properties of Suc-GPLGP-MCAase and dipeptidyl-aminopeptidase in mouse calvaria-derived osteoblastic cells (MC3T3-E1). Calcif Tissue Int 37, 183–188 (1985). https://doi.org/10.1007/BF02554839

Background

Product Data Sheet

Suc-Gly-Pro-Leu-Gly-Pro-AMC, a highly sensitive, fluorogenic substrate for thimet oligopeptidase as well as for post-proline cleaving enzyme (prolyl endopeptidase) [1].

The FAP activity in human plasma was measured using succinyl-pentapeptide composed of SUch-Gly-pro-Leu-Gly-pro-AMC, which contains two FAP cleavage sites.Only the cleavage between the last proline residue and the AMC group releases the fluorescent AMC group, resulting in a fluorescence reading, and the FAP activity of the measured samples ranges from 1.3 to 7 nM/min/ μL[2].

When each cell suspension was reacted with BODIPY FL casein and seven kinds of peptide-MCA substrates, respectively, a remarkable difference in hydrolytic activities toward Suc-GPLGP-MCA, a substrate toward collagenase-like peptidase, was observed between the constructs: Lc-Triad-displaying cells showed higher catalytic activity than Lc-WT-displaying cells[5].

In Male BALB/c mice □using Suc-Gly-Pro-Leu-Gly-Pro-AMC test□no significant accumulations of other proteinases, such as matrix metalloproteinases, cathepsin D, and serine proteinases, were determined[3].Suc-GPLGP-MCA is hydro- lyzed at the Leu-Gly bond by CL-peptidase. The CL-peptidase activity in synovial fluid was significantly higher in patients with rheumatoid arthritis (RA) than in patients with osteoarthritis (OA) and in arthropathy-free controls[4].

References:
[1]: Kojima K, Kinoshita H, et,al. A new and highly sensitive fluorescence assay for collagenase-like peptidase activity. Anal Biochem. 1979 Nov 15;100(1):43-50. doi: 10.1016/0003-2697(79)90106-4. PMID: 232383.
[2]: Zhen EY, Jin Z, et,al. Circulating FGF21 proteolytic processing mediated by fibroblast activation protein. Biochem J. 2016 Mar 1;473(5):605-14. doi: 10.1042/BJ20151085. Epub 2015 Dec 3. PMID: 26635356; PMCID: PMC4764976.
[3]: Kakegawa H, Matano Y, et,al. Significant accumulations of cathepsin B and prolylendopeptidase in inflammatory focus of delayed-type hypersensitivity induced by Mycobacterium tuberculosis in mice. Biochem Biophys Res Commun. 2004 Mar 26;316(1):78-84. doi: 10.1016/j.bbrc.2004.01.176. PMID: 15003514.
[4]:Ito A, Hagihara M, et,al. Collagenase-like (CL) peptidase activity in synovial fluid from patients with rheumatoid arthritis. Clin Chim Acta. 1987 Dec;170(2-3):291-6. doi:

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10.1016/0009-8981(87)90139-2. PMID: 2830060.
[5]:Okochi N, Kato-Murai M, et,al. Design of a serine protease-like catalytic triad on an antibody light chain displayed on the yeast cell surface. Appl Microbiol Biotechnol. 2007 Dec;77(3):597-603. doi: 10.1007/s00253-007-1197-0. Epub 2007 Sep 27. PMID: 17899065.

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