Product Data Sheet

Product Name: Ac-Ile-Glu-Pro-Asp-pNA

Cat. No.: GA20580

Chemical Properties

Cas. No.	216757-29-8		
Formula	$C_{28}H_{38}N_6O_{11}$	M.Wt	634.64
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	Ac-Ile-Glu-Pro-Asp-pNA		

Protocol

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1. □□□□

□1□□□□□□DMSO□□Ac-Ile-Glu-Pro-Asp-pNA□□□□□1 mM□
□□:□□□□□□□□□□□□-20℃□□□□□□□□□□□□□

□2□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□0.2mM□
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2. □□Ac-Ile-Glu-Pro-Asp-pNA□□□□□□□□B□□□□□[1](□□□□,□□□□)
□1□□□□□□□10μL□□□□□□□□□□96□□□□
□2□□□□□□□□90μL□Ac-Ile-Glu-Pro-Asp-pNA□□□□□□□0.2mM□
□3□□□□□□□□0□□□90□□□□□□□405 nm□□□□□□□OD□□□□OD□□□□□□□□□□□□□□□□□□□□□□□□

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References:

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[1] 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.

Background

Ac-Ile-Glu-Pro-Asp-pNA (Ac-IEPD-pNA) is a colorimetric substrate for granzyme B (GzmB) and caspase-8 [1, 2]. Granzyme B and caspase-8 preferentially bind and cleave the Ile-Glu-Pro-Asp (IEPD) peptide sequence, and the catalytic activity of the enzyme is indirectly measured by detecting the release of p-nitroaniline (pNA), which is measured by the optical density (OD) at 405 nm[3]. Granzyme B is a serine protease found in cytotoxic T cells and NK cells, which cleaves and activates several caspases involved in apoptosis[4]. Caspase-8 is a caspase protein that participates in the signal transduction of death receptors of the tumor necrosis factor receptor family and is also essential for the induction of the transcription factor NF-κB[5]. Ac-Ile-Glu-Pro-Asp-pNA is recommended as a potential granzyme B target and the optimal P4-P1 substrate sequence for granzyme B.

The structural and group characteristics of Ac-Ile-Glu-Pro-Asp-pNA are as follows:

(1) N-acetyl group (Ac): protects the N-terminus of the amino acid to prevent nonspecific reactions.

(2) Amino acid sequence (Ile-Glu-Pro-Asp): This specific sequence mimics certain natural substrates and helps to study the specificity of proteases for different substrates.

(3) p-Nitroaniline (pNA): It is a common chromogenic group that releases a yellow product with light-absorbing properties after hydrolysis, which is convenient for monitoring enzyme reactions through spectral measurement.

References:

[1]Ewen C, Kane K P, Shostak I, et al. A novel cytotoxicity assay to evaluate antigen-specific CTL responses using a colorimetric substrate for Granzyme B[J]. Journal of immunological methods, 2003, 276(1-2): 89-101.
[2]Almeida S, Domingues A, Rodrigues L, et al. FK506 prevents mitochondrial-dependent apoptotic cell death induced by 3-nitropropionic acid in rat primary cortical cultures[J]. Neurobiology of disease, 2004, 17(3): 435-444.

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[3]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.
[4]Rousalova I, Krepela E. Granzyme B-induced apoptosis in cancer cells and its regulation[J]. International journal of oncology, 2010, 37(6): 1361-1378.
[5]Chun H J, Zheng L, Ahmad M, et al. Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency[J]. Nature, 2002, 419(6905): 395-399.

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