

Product Data Sheet

Product Name: Ac-YVAD-AFC

Cat. No.: GC13326

Chemical Properties

Cas. No. 219137-85-6

Chemical Name (4S,7S,10S,13S)-4-(4-hydroxybenzyl)-7-isopropyl-10-methyl-2,5,8,11-tetraoxo-13-((2-oxo-4-(trifluoromethyl)-2H-chromen-7-yl)carbonyl)-3,6,9,12-tetraazapentadecan-15-oic acid

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Formula C₃₃H₃₆F₃N₅O₁₀

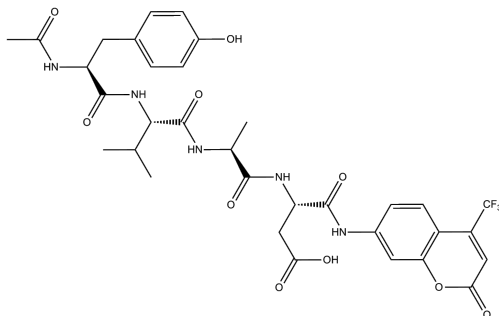
M.Wt 719.66

Solubility 10mg/ml in DMSO; 1mg/ml in DMF Storage Store at -20°C, protect from light

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

Ac-YVAD-AFC Assay for caspase-1 Activity in Cells or Mice^[1-2]

This experimental protocol is compiled based on the research content and for reference only. Please adjust according to actual needs.

(1) Reagent preparation: dissolve Ac-YVAD-AFC in dimethyl sulfoxide (DMSO) to prepare a 10mM

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stock solution. Further dilute to a working concentration (50 μ M) before use. Prepare a buffer containing 100mM HEPES (pH=7.5), 10% glycerol, and 10mM dithiothreitol (DTT).

(2) Sample preparation: for cell samples, lyse cells in RIPA buffer (containing 50mM Tris-HCl (pH=8.0), 150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS). Centrifuge at 15000g for 15 minutes. Collect the supernatant as the enzyme source. For mouse in vivo samples, collect mouse hippocampus or cortex tissue. Homogenize the tissue in 5 volumes/weight of RIPA buffer (containing 50mM Tris-HCl (pH=7.4), 1% NP-40, 150mM NaCl, 0.25% Na-deoxycholate, 1mM EDTA, 1mM Na₃VO₄, 1mM NaF, 1mM PMSF, and 10 μ l/ml protease inhibitor mixture) using a tissue homogenizer on ice. Centrifuge at 20000g for 20 minutes. Collect the supernatant as the enzyme source.

(3) caspase-1 activity assay: add enzyme source supernatant and final concentration of 50 μ M Ac-YVAD-AFC substrate to the reaction buffer (100mM HEPES (pH=7.5), 10% glycerol, 10mM DTT). Incubate the total reaction system at 37°C for 2 hours. Measure the fluorescence intensity at an excitation wavelength of 400nm and an emission wavelength of 505nm using a multifunctional microplate reader.

Precautions:

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

References:

- [1] Flores J, Noel A, Foveau B, et al. Caspase-1 inhibition alleviates cognitive impairment and neuropathology in an Alzheimer's disease mouse model. Nat Commun. 2018 Sep 25;9(1):3916.
- [2] Furuoka M, Ozaki K, Sadatomi D, et al. TNF- α Induces Caspase-1 Activation Independently of Simultaneously Induced NLRP3 in 3T3-L1 Cells. J Cell Physiol. 2016;9999:1-7.

Background

Ac-YVAD-AFC is a fluorescent peptide substrate for caspase-1. Caspase-1 can recognize the YVAD sequence and cut the Asp-AFC bond to release a 7-amino-4-trifluoromethylcoumarin fluorescent signal, with an excitation wavelength of 400nm and an emission wavelength of 505nm. Ac-YVAD-AFC can be used for studies on inflammasome, pyroptosis, and pro-IL-1 β and pro-IL-18 maturation^[1-4].

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- [1] Zhu H, Yuan Z, Xu H, et al. Characterization of the Apoptotic and Antimicrobial Activities of Two Initiator Caspases of Sea Cucumber *Apostichopus japonicus*. *Genes* (Basel). 2024 Apr 25;15(5):540.
- [2] Hao K, Yuan L, Yu C, et al. *Paralichthys olivaceus* GSDME-mediated pyroptosis is regulated by multiple caspases in different manners. *Fish Shellfish Immunol*. 2024 Dec;155:110002.
- [3] Jia C, Zhang M, Wu X, et al. HERV-W Env Induces Neuron Pyroptosis via the NLRP3-CASP1-GSDMD Pathway in Recent-Onset Schizophrenia. *Int J Mol Sci*. 2025 Jan 9;26(2):520.
- [4] Guo RF, Huber-Lang M, Wang X, et al. Protective effects of anti-C5a in sepsis-induced thymocyte apoptosis. *J Clin Invest*. 2000 Nov;106(10):1271-80.

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