

Product Data Sheet

Product Name: Z-Gly-Gly-Arg-AMC acetate

Cat. No.: GC37965

Chemical Properties

Cas. No. 2070009-61-7

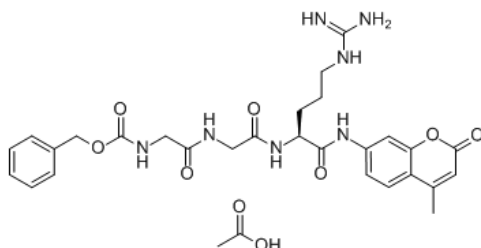
Formula $C_{30}H_{37}N_7O_9$ M.Wt 639.66

Solubility Water : ≥ 60 mg/mL (93.80 mM) 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

Detection of thrombin activity based on Z-Gly-Gly-Arg-AMC acetate^[1]

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

1. Solution preparation

(1) Dissolve the Z-Gly-Gly-Arg-AMC acetate solid in an appropriate amount of DMSO to prepare a 50mM stock solution. Store stock solution at -20°C and avoid repeated freezing and thawing.

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

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(2) Dilute the appropriate Z-Gly-Gly-Arg-AMC acetate stock solution in PBS to prepare a 2.5mM working solution. The working solution should be prepared immediately and used up promptly.

(3) Take an appropriate CaCl_2 solution and dissolve in the detection buffer (containing 20mM HEPES, 145mM NaCl, pH 7.5) to prepare the activation buffer.

2. Thrombin generation assay

(1) Mix the plasma with the Z-Gly-Gly-Arg-AMC acetate stock solution at a volume ratio of 4:1, and place the mixture in the wells of a flat-bottom 96-well plate. Add 100 μl of the mixture to each well. Add 20 μl of activation buffer to each well to trigger the clotting reaction. The final concentration of Z-Gly-Gly-Arg-AMC acetate is 400 μM .

(2) Fluorescence is continuously measured at 37°C using a fluorescence plate reader (Ex/Em=355/460nm) for a period of 30min with a read every 3min.

3. Notes for Attention

(1) Z-Gly-Gly-Arg-AMC acetate is photosensitive and the operation process should be light-protected.

(2) To avoid bubbles, the reading should be taken within 10 seconds after the activation buffer (containing CaCl_2) is added.

References:

[1] Tarandovskiy I D, Artemenko E O, Panteleev M A, et al. Antiplatelet agents can promote two-peaked thrombin generation in platelet rich plasma: mechanism and possible applications[J]. PLoS One, 2013, 8(2): e55688.

Background

Z-Gly-Gly-Arg-AMC acetate is a specific thrombin fluorescent substrate with $\lambda_{\text{exc}}=355\text{nm}$, $\lambda_{\text{em}}=460\text{nm}$ ^[1]. Z-Gly-Gly-Arg-AMC acetate acts as the reference substrate in the Calibrated Automated Thrombogram (CAT) for measuring endogenous thrombin potential (ETP) in platelet-rich (PRP) or platelet-poor plasma (PPP)^[2]. Z-Gly-Gly-Arg-AMC acetate is employed in 96-well format for rapid identification and potency ranking of direct thrombin inhibitors or protease-activated receptor antagonists^[3].

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- [2] Machlus K R, Colby E A, Wu J R, et al. Effects of tissue factor, thrombomodulin and elevated clotting factor levels on thrombin generation in the calibrated automated thrombogram[J]. Thrombosis and haemostasis, 2009, 102(11): 936-944.
- [3] Kale S S, Bergeron-Brlek M, Wu Y, et al. Thiol-to-amine cyclization reaction enables screening of large libraries of macrocyclic compounds and the generation of sub-kilodalton ligands[J]. Science advances, 2019, 5(8): eaaw2851.

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