

Product Data Sheet

Product Name: 4-Azido-L-phenylalanine (p-Azidophenylalanine)

Cat. No.: GC30007

Chemical Properties

Cas. No. 33173-53-4

SMILES O=C(O)[C@@H](N)CC1=CC=C(N=[N+]=[N-])C=C1

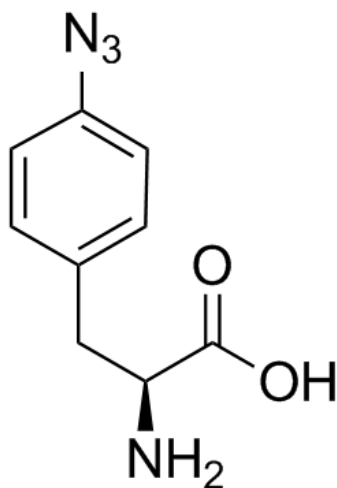
Formula C9H10N4O2 M.Wt 206.2

Solubility Water : 6.2 mg/mL (30.07 mM; ultrasonic and adjust pH to 6 with HCl); DMSO : 6 mg/mL (29.10 mM; ultrasonic and adjust pH to 6 with HCl) 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

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

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

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Unnatural amino acid labeling in living cells via azide-alkyne click chemistry [1].

1. Cell culture:

At 24h before transfection, HEK293T cells were cultured on 18mm poly-lysine-coated glass coverslips, the cell culture medium was changed to supplemented with 0.65mM 4-Azido-L-phenylalanine (p-Azidophenylalanine). growth medium.

2. Transfection experiments

pIRE4-Azi plasmid (1:1 w/w) was transfected into cells using Lipofectamine 3000 reagent. After 24h, the growth medium was refreshed with 0.65mM 4-Azido-L-phenylalanine (p-Azidophenylalanine) and the cells were cultured for an additional 24h (48h total expression). 30min before labeling, 4-azido-L-phenylalanine supplemented growth medium was removed, and cells were washed twice by extracellular buffer solution containing 128mM NaCl, 2mM KCl, 2.5mM CaCl₂, 1.2mM MgCl₂, 10mM sucrose, 10mM HEPES, and pH7.4 and were kept in growth medium without 4-azido-L-phenylalanine.

3. Unnatural amino acid labeling in living cells via azide-alkyne click chemistry

3.1 Preparation of stock solutions

Sulfo-Cyanine3.5 alkyne (catalog number: GC59209) alkyne dyes 10mM in DMSO, BTES (Click Chemistry Tools) 50mM, copper sulfate (catalog number: GB57835) 20mM, Aminoguanidine (catalog number: GC15727) 100mM, and (+)-sodium L-ascorbate (catalog number: GC13616) 100mM in ultrapure distilled water.

3.2 Click on Chemical Reaction

A: In 656μl of extracellular buffer solution, Cy3.5 were mixed to a final concentration of 20μM. Fresh copper sulfate solution and BTES (1:5 molar ratio) solution were premixed and then added to a final concentration of 150μM. Next, add Aminoguanidine to a final concentrate. The total labeling volume is 0.7mL;

B: Incubate the completed labeling mixture at 4°C for 8 minutes, then at room temperature for 2 minutes and in the dark before adding to the cells. Wash the cells before adding the labeling mixture. During labeling, keep the cells in the dark at 37°C and 5% CO₂ for 10-15 minutes. After labeling, wash the cells twice with extracellular buffer. Cy3.5-labeled products can be observed at 591/604nm using confocal microscopy.

References:

[1].Schamber M R, Vafabakhsh R. Mechanism of sensitivity modulation in the calcium-sensing receptor via electrostatic tuning[J]. Nature communications, 2022, 13(1): 2194.

Background

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4-Azido-L-phenylalanine (p-Azidophenylalanine) is an unnatural amino acid analog of L-phenylalanine that contains an azide moiety. 4-Azido-L-phenylalanine (p-Azidophenylalanine)-labeling is a fast, sensitive, and non-radioactive alternative to the traditional technique for detecting nascent protein synthesis.

4-Azido-L-phenylalanine (p-Azidophenylalanine) is randomly incorporated into synthesizing protein instead of phenylalanine during translation. The resulting azide-labeled full-length proteins can be detected via copper-catalyzed click reaction (with fluorescent or biotin-labeled alkynes) or copper-free click reaction (with cycloalkynes) and used for subsequent microscopic imaging or purification tasks.

References:

[1].Peng Y F. Methodology to Site-Specifically Label SBDS Domains to Monitor Dynamics of 60S Ribosomal Subunit Interactions[D]. Saint Louis University, 2023.

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