OABK hydrochloride

Cat. No.: HY-100825

CAS No.: 1984862-48-7

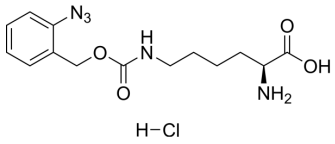
Molecular Formula: $C_{14}H_{20}ClN_5O_4$

Molecular Weight: 357.79

Target: Others

Pathway: Others

Storage: 4°C, sealed storage, away from moisture
* In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 25 mg/mL (69.87 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.7949 mL	13.9747 mL	27.9494 mL
	5 mM	0.5590 mL	2.7949 mL	5.5899 mL
	10 mM	0.2795 mL	1.3975 mL	2.7949 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (6.99 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (6.99 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (6.99 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

OABK hydrochloride is a small-molecule switch that can be used to control protein activity. OABK (hydrochloride) is a click chemistry reagent, it contains an Azide group and can undergo copper-catalyzed azide-alkyne cycloaddition reaction (CuAAc) with molecules containing Alkyne groups. Strain-promoted alkyne-azide cycloaddition (SPAAC) can also occur with molecules containing DBCO or BCN groups.

In Vitro

A small-molecule switch for the activation of protein function through the site-specific incorporation of an ortho-azidobenzyloxycarbonyl lysine (OABK). The amino acid OABK is synthesized readily in three steps from 2-azidobenzyl alcohol via a succinimidyl carbonate. Deprotection results in the formation of lysine and, when OABK is incorporated into a protein, the formation of active wild-type protein. Genetically encoded OABK in conjunction with small-molecule activation

allows for the conditional regulation of intracellular protein maturation. Incorporation of OABK (0.5 mM) at position K85 of EGFP inhibits fluorophore formation until the native lysine is generated through small-molecule activation (the model is based on Protein Data Bank (PDB). Introducing OABK at position K206 inhibits FLuc enzymatic activity by restricting the access of adenosine triphosphate (ATP) to the active site, until the enzyme is deprotected and activated through phosphine treatment. The incorporation of OABK into FLuc blocks the luciferase activity in the absence of small-molecule activation, as determined by a Bright-Glo luciferase assay[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay [1]

HEK293T cells are plated at 100,000 cells per well (400 µL) into a poly-D-lysine-coated eight-well chamber slide. At 75% confluency, cells are co-transfected with pEGFP-K85TAG-mCherry or pEGFP-K29TAG-SatB1-mCherry and pOABKRS-4PylT (200 ng of each plasmid) using linear PEI (3 µL, 0.323 mg/mL). After 20 hours of incubation at 37°C and 5% CO_2 in DMEM with 10% FBS in the presence of OABK (0.25 mM), the cells are washed three times with phenol-red-free DMEM (200 µL), followed by three hours of incubation to remove any non-incorporated OABK. Before small-molecule activation, the cells are focused using the Texas Red channel, and imaged with a Nikon A1 confocal microscope (×40 oil objective, ×2 zoom, fluorescein isothiocyanate (ex=488 nm) and Texas Red (ex=560 nm) channels)[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Luo J, et al. Small-molecule control of protein function through Staudinger reduction. Nat Chem. 2016 Nov;8(11):1027-1034.

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