EN219-alkyne

Cat. No.: HY-115715A

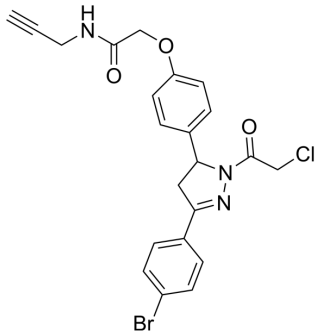
Molecular Formula: $C_{22}H_{19}BrClN_3O_3$

Molecular Weight: 488.76

Target: Others

Pathway: Others

Storage: Please store the product under the recommended conditions in the Certificate of Analysis.



BIOLOGICAL ACTIVITY

Description

EN219-alkyne is an alkyne-functionalized EN219 probe. EN219 (HY-P0287A) is a moderately selective synthetic covalent ligand against an N-terminal cysteine (C8) of RNF114 with an IC_{50} of 470 nM. EN219 inhibits RNF114-mediated autoubiquitination and p21 ubiquitination[1][2]. EN219-alkyne is a click chemistry reagent, it contains an Alkyne group and can undergo copper-catalyzed azide-alkyne cycloaddition (CuAAc) with molecules containing Azide groups.

In Vitro

EN219-alkyne probe labeling in situ and pulldown studies (in 231MFP cells for example)[1]
1.Treat 231MFP cells with either DMSO vehicle or 50 μM EN219-alkyne probe for 90 min.
2.Collect cells in PBS and lysed by sonication.
3.Prepare Western blotting samples:
Aliquot the lysate (1 mg of protein in 500 μL) per sample, and then add: 10 μL of 5 mM biotin picolylazide and 50 μL of click reaction mix (three parts TBTA 5 mM TBTA in butanol:DMSO (4:1, v/v), one part 50 mM Cu(II)SO4 solution and one part 50 mM TCEP).
4.Incubate samples for 1 h at room temperature with gentle agitation.
5.After CuAAC, precipitate proteomes by centrifugation at 6,500 g and washed twice in ice-cold methanol (500 μL).
6.Spun samples in a prechilled (4℃) centrifuge at 6,500 g for 4 min, aspiration of excess methanol and subsequent reconstitution of protein pellet in 250 μL PBS containing 1.2% SDS by probe sonication.
7.Denature the proteomes at 90℃ for 5 min, precipitate the insoluble components by centrifugation at 6,500g, and dilute soluble proteome in 1.2 ml PBS (the final concentration of SDS in the sample was 0.2%) to a total volume of 1450 μL, with 50 μL reserved as input.
8.Add pre-washed 85 μL 50% streptavidin agarose bead slurry to each sample, and incubate samples overnight at room temperature with gentle agitation.
9.Aspirate supernatant from each sample after spinning beads at 6,500 g for 2 min at room temperature.
10.Transfer beads to spin columns and wash beads three times with PBS. To elute, boil beads 5 min in 50 μL LDS sample buffer. Collect eluents after centrifugation for immunoblotting analysis.

EN219 (1 μM; 90 min) interacts with RNF114 C8, TUBB1 C201, HSPD1 C442, and HIST1H3A C97 demonstrated by isotopic tandem orthogonal proteolysisABPP (isoTOP-ABPP) analysis[2].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Luo M, et al. Chemoproteomics-enabled discovery of covalent RNF114-based degraders that mimic natural product function. Cell Chem Biol. 2021 Apr 15;28(4):559-566.e15.

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