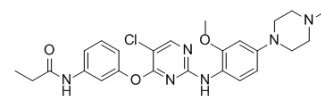


## WZ4003

Cat. No.:	HY-15802		
CAS No.:	1214265-58-3		
Molecular Formula:	C <sub>25</sub> H <sub>29</sub> ClN <sub>6</sub> O <sub>3</sub>		
Molecular Weight:	496.99		
Target:	AMPK		
Pathway:	Epigenetics; PI3K/Akt/mTOR		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month



### SOLVENT & SOLUBILITY

In Vitro	DMSO : 33.33 mg/mL (67.06 mM; Need ultrasonic)				
		Solvent Concentration	Mass 1 mg	5 mg	10 mg
	Preparing Stock Solutions	1 mM	2.0121 mL	10.0606 mL	20.1211 mL
		5 mM	0.4024 mL	2.0121 mL	4.0242 mL
10 mM		0.2012 mL	1.0061 mL	2.0121 mL	
Please refer to the solubility information to select the appropriate solvent.					
In Vivo	1. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (5.03 mM); Clear solution				
	2. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.5 mg/mL (5.03 mM); Clear solution				

### BIOLOGICAL ACTIVITY

Description	WZ4003 is the first potent and highly specific NUA1 kinase inhibitor with IC <sub>50</sub> of 20 nM/100 nM for NUA1 (ARK5)/NUAK2, without significant inhibition on other 139 kinases.	
IC <sub>50</sub> & Target	NUAK1 20 nM (IC <sub>50</sub> )	NUAK2 100 nM (IC <sub>50</sub> )
In Vitro	WZ4003 (3-10 μM) markedly suppresses NUA1-mediated MYPT1 phosphorylation, in HEK-293 cells expressing wild-type NUA1. Moreover, WZ4003 (10 μM) inhibits MYPT1 Ser445 phosphorylation as well as cell migration, invasion and proliferation to a similar extent as knock out in MEFs or knock down in U2OS cells of NUA1 <sup>[1]</sup> . WZ4003 also exhibits a high, specific affinity to the L858R/T790M mutant EGFR, while a significantly reduced cellular IC <sub>50</sub> against T790M containing Ba/F3	

cells<sup>[2]</sup>.

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

## PROTOCOL

### Kinase Assay <sup>[1]</sup>

In vitro activities of purified GST-NUAK1 and GST-NUAK1[A195T] are measured using Cerenkov counting of incorporation of radioactive <sup>32</sup>P from [ $\gamma$ -<sup>32</sup>P]ATP into Sakamototide substrate peptide. Reactions are carried out in a 50  $\mu$ L reaction volume for 30 min at 30°C and reactions are terminated by spotting 40  $\mu$ L of the reaction mix on to P81 paper and immediately immersing in 50 mM orthophosphoric acid. Samples are washed three times in 50 mM orthophosphoric acid followed by a single acetone rinse and air drying. The kinase-mediated incorporation of [ $\gamma$ -<sup>32</sup>P]ATP into Sakamototide is quantified by Cerenkov counting. One unit of activity is defined as that which catalysed the incorporation of 1 nmol of [<sup>32</sup>P]phosphate into the substrate over 1 h.

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

### Cell Assay <sup>[1]</sup>

Cell proliferation assays are carried out colorimetrically in 96-well plates. Initially, 2000 cells per well are seeded for U2OS cells and 3000 cells per well are seeded for MEFs. The proliferation assays are carried out over 5 days in the presence or absence of 10  $\mu$ M HTH-01-015 or WZ4003.

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

## CUSTOMER VALIDATION

- Cancer Discov. 2018 May;8(5):632-647.
- J Cell Biol. 2019 Apr 1;218(4):1369-1389.
- Commun Biol. 2021 Mar 25;4(1):399.
- Harvard Medical School LINCS LIBRARY

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## REFERENCES

[1]. Banerjee S, et al. Characterization of WZ4003 and HTH-01-015 as selective inhibitors of the LKB1-tumour-suppressor-activated NUA1 kinases. *Biochem J.* 2014 Jan 1;457(1):215-25.

[2]. Zhou W, et al. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. *Nature.* 2009 Dec 24;462(7276):1070-4

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

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