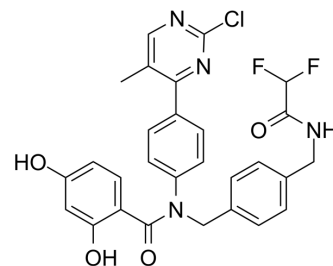


## VER-246608

<b>Cat. No.:</b>	HY-12492		
<b>CAS No.:</b>	1684386-71-7		
<b>Molecular Formula:</b>	C <sub>28</sub> H <sub>23</sub> ClF <sub>2</sub> N <sub>4</sub> O <sub>4</sub>		
<b>Molecular Weight:</b>	553		
<b>Target:</b>	PDHK		
<b>Pathway:</b>	Metabolic Enzyme/Protease		
<b>Storage:</b>	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



### SOLVENT & SOLUBILITY

<b>In Vitro</b>	DMSO : 100 mg/mL (180.83 mM; Need ultrasonic)			
		Solvent Concentration	Mass	
			1 mg	5 mg
			10 mg	
<b>Preparing Stock Solutions</b>	<b>1 mM</b>	1.8083 mL	9.0416 mL	18.0832 mL
	<b>5 mM</b>	0.3617 mL	1.8083 mL	3.6166 mL
	<b>10 mM</b>	0.1808 mL	0.9042 mL	1.8083 mL
Please refer to the solubility information to select the appropriate solvent.				
<b>In Vivo</b>	1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.5 mg/mL (4.52 mM); Clear solution  2. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.5 mg/mL (4.52 mM); Clear solution			

### BIOLOGICAL ACTIVITY

<b>Description</b>	VER-246608 is a potent and ATP-competitive inhibitor of pyruvate dehydrogenase kinase (PDK) with IC <sub>50</sub> s of 35 nM, 40 nM, 84 nM, and 91 nM for PDK-1, PDK-3, PDK-2, and PDK-4, respectively.
<b>IC<sub>50</sub> &amp; Target</b>	IC <sub>50</sub> : 35 nM (PDK-1), 40 nM (PDK-3), 84 nM (PDK-2), 91 nM (PDK-4) <sup>[1]</sup>
<b>In Vitro</b>	VER-246608 is a novel pan-isoform ATP competitive inhibitor of PDK. VER-246608 demonstrates similar potency across all four PDK isoforms in a DELFIA-based enzyme functional assay in the sub 100 nM range. In terms of cellular biomarker modulation, VER-246608 suppresses the phosphorylation of the Ser <sup>293</sup> residue of E1α (phosphorylated by all four PDK isozymes) with IC <sub>50</sub> values of 266 nM. Treatment of PC-3 cells with 9 μM and 27 μM VER-246608 results in a 21% and 42% reduction, respectively, in media L-lactate levels following a 1 h incubation. VER-246608 also decreases D-glucose

consumption at the same concentrations that result in reduced L-lactate production. An approximately 50% reduction in spheroid volume is achieved at concentrations of 10  $\mu\text{M}$  and above, suggesting an increase in VER-246608 potency compared to monolayer growth<sup>[1]</sup>.

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

## PROTOCOL

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

DELFLIA assay reagents (assay buffer, wash buffer, enhancement solution and anti-rabbit IgG-Eu-N1 secondary antibody) and plates are used. Test compounds are subjected to a 10 point tripling dilution in DMSO, diluted in MOPS buffer (60 mM MOPS pH7.2, 15 mM Magnesium acetate, 60 mM KCl) and added to the enzyme mix (10 nM PDK-1, 2 and 3 or 20 nM PDK-4, 300 nM E1, 0.1 mg/mL BSA, 1 mM DTT) in 96-well V-bottom plates. The reaction is initiated by the addition of ATP to a final concentration of 5  $\mu\text{M}$  followed by a 1 h incubation at 30°C. The reaction is then stopped by the addition of STOP solution (50 mM Carbonate-Bicarbonate Buffer, pH 9.6), and then transferred to 96 well DELFLIA yellow plates. The plates are then sealed and incubated o/n at 4°C. Detection and quantification of p(Ser<sup>293</sup>)E1 $\alpha$  levels is then achieved through incubation with anti-p(Ser<sup>293</sup>)E1 $\alpha$  primary antibody followed by anti-rabbit secondary IgG-Eu-N1 antibody and addition of enhancement solution. The time-resolved fluorescent signal is then measured using a Victor2 plate reader. The data is fitted by non-linear regression using XLFIT4 within a custom ABASE (IDBS) protocol in order to determine IC<sub>50</sub> values<sup>[1]</sup>.

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

Compound cytotoxicity is determined using the Sulforhodamine B assay for cells cultured as a monolayer. For spheroid growth experiments, PC-3 cells are seeded (500 cells/well) into 96 well round bottom plates in RPMI-1640 media containing 2.5% (w/v) Matrigel. The resultant spheroids are treated with VER-246608 (2.5, 5, 10, 20, and 40  $\mu\text{M}$ ) 48 h post-seeding. Spheroid volumes are determined by obtaining diameter measurements from images taken on a Zeiss Axiovert 200 M inverted microscope using the axiovision software<sup>[1]</sup>.

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

## REFERENCES

[1]. Moore JD, et al. VER-246608, a novel pan-isoform ATP competitive inhibitor of pyruvate dehydrogenase kinase, disrupts Warburg metabolism and induces context-dependent cytostasis in cancer cells. *Oncotarget*. 2014 Dec 30;5(24):12862-76.

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

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