MLCK inhibitor peptide 18

Cat. No.:	HY-P1029
CAS No.:	224579-74-2
Molecular Formula:	$C_{60}H_{105}N_{23}O_{11}$
Molecular Weight:	1324.62
Sequence:	Arg-Lys-Lys-Tyr-Lys-Tyr-Arg-Arg-Lys-NH2
Sequence Shortening:	RKKYKYRRK-NH2
Target:	Myosin; CaMK; Autophagy
Pathway:	Cytoskeleton; Neuronal Signaling; Autophagy
Storage:	Sealed storage, away from moisture Powder -80°C 2 years -20°C 1 year * In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture)

RKKYKYRRK-NH_2

SOLVENT & SOLUBILITY

In Vitro

H_2O : 100 mg/mL (75.49 mM; Need ultrasonic)

Preparing Stock Solutions

Concentration \ Solvent \ Mass	1 mg	5 mg	10 mg
1 mM	0.7549 mL	3.7747 mL	7.5493 mL
5 mM	0.1510 mL	0.7549 mL	1.5099 mL
10 mM	0.0755 mL	0.3775 mL	0.7549 mL

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

In Vivo

1. Add each solvent one by one: PBS
   Solubility: 100 mg/mL (75.49 mM); Clear solution; Need ultrasonic

BIOLOGICAL ACTIVITY

Description

MLCK inhibitor peptide 18 is a myosin light chain kinase (MLCK) inhibitor with an IC_{50} of 50 nM, and inhibits CaM kinase II only at 4000-fold higher concentrations.

IC_{50} & Target

CaMK II

In Vitro

MLCK inhibitor peptide 18 has a selective effect on peptide substrate utilization by MLCK, does not interfere with kinase activation by CaM, does not have significant inhibitory activity with the closely related CaMPKII, and does not inhibit PKA[1].

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

PROTOCOL

Kinase Assay [1]

All assays are done in a final volume of 50 µL. Each point is tested in duplicate. The standard assay conditions are 50 mM HEPES, 0.2 mM [γ-^{32}P]ATP (specific activity 200−300 cpm/pmol), 1 mM DTT, 1 mg/mL bovine serum albumin, 5 mM $MgCl_2$, 150 mM KCl, 15 mM NaCl, pH 7.5, with or without inhibitors. Synthetic substrate concentrations, KKRPQRATSNVFAM-NH2 for MLCK, PLRRTLSVAA-NH2 for CaMPKII, and LRRASLG for PKA, are 20 µM. The reactions are initiated with the addition of 5 µL of enzyme (PKA) or enzyme:CaM complex (for MLCK and CaMPKII). Enzyme:CaM complexes are preformed by incubation of enzyme in ice for 1 min in the presence of 1 µM CaM and 5 µM $CaCl_2$ (for MLCK) or 30 µM CaM and 2 mM $CaCl_2$ (for CaMPKII). Assay conditions are 20 min, 25°C for MLCK; 8 min, 25°C for CaMPKII; 6 min, 25°C for PKA. Aliquots of each reaction tube are spotted into phosphocellulose filter paper. Filters are washed in 75 mM phosphoric acid solution, followed by 95% ethanol, dried, and placed into vials containing 10 mL of Ecoscint O. Radioactivity is determined by counting vials in a Beckman 6500 liquid scintillation counter. No quenching corrections are applied. Data are transformed as percent of the maximal enzyme activity, where enzyme activity in the absence of inhibitors is taken as 100%. ICsub>50 values are calculated by a linear regression data analysis on data points corresponding to 15%−85% of maximal activity. This analysis is done using InPlot version 4.0.

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CUSTOMER VALIDATION

- Cell Mol Gastroenterol Hepatol. 2021;11(3):683-696.
- J Cell Sci. 2022 Jun 6;jcs.259692.

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REFERENCES

[1]. Lukas TJ, et al. Identification of novel classes of protein kinase inhibitors using combinatorial peptide chemistry based on functional genomics knowledge. J Med Chem. 1999 Mar 11;42(5):910-9.

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