产品名称：KU-0063794
产品别名：KU-0063794

生物活性：

Description	KU-0063794 is a potent and specific mTOR inhibitor, inhibiting both the mTORC1 and mTORC2 complexes with IC50s of 10 nM.		
IC_{50} & Target	mTORC1	mTORC2	
	10 nM (IC_{50})	10 nM (IC_{50})	
In Vitro	Ku-0063794 is cell permeant, suppresses activation and hydrophobic motif phosphorylation of Akt, S6K and SGK, but not RSK (ribosomal S6 kinase), an AGC kinase not regulated by mTOR. Ku-0063794 also inhibits phosphorylation of the T-loop Thr308 residue of Akt phosphorylated by PDK1 (3-phosphoinositide-dependent protein kinase-1). Ku-0063794 induces a much greater dephosphorylation of the mTORC1 substrate 4E-BP1 (eukaryotic initiation factor 4E-binding protein 1) than rapamycin, even in mTORC2-deficient cells, suggesting a form of mTOR distinct from mTORC1, or mTORC2 phosphorylates 4E-BP1. Ku-0063794 also suppresses cell growth and induced a G1-cell-cycle arrest[1]. Ku0063794 does not alter nuclear phospho-Mst1-Thr-120 levels in LNCaP cell nuclei, whereas Ku0063794 or CCI-779 increases phospho-Mst1-Thr-120 levels in C4-2 cell nuclei[2]. The combination of GDC-0941 and KU0063794 inhibits the phosphorylation of 4EBP1 and S6 to a similar extent to that caused by single agent NVP-BEZ235 in HCT116, DLD1 and HT29 cell lines[3].		

Solvent&Solubility

In Vitro:

DMSO : 16.67 mg/mL (35.81 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.1480 mL	10.7402 mL	21.4804 mL
	5 mM	0.4296 mL	2.1480 mL	4.2961 mL
	10 mM	0.2148 mL	1.0740 mL	2.1480 mL

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 **In Vitro** 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 1.67 mg/mL (3.59 mM); Clear solution

此方案可获得 ≥ 1.67 mg/mL (3.59 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 16.699999 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline)

Solubility: ≥ 1.67 mg/mL (3.59 mM); Clear solution

	此方案可获得 ≥ 1.67 mg/mL (3.59 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 16.699999 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 1.67 mg/mL (3.59 mM); Clear solution 此方案可获得 ≥ 1.67 mg/mL (3.59 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 16.699999 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Garcia-Martinez et al. Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). Biochem.J. (2009)421 29. [2]. Collak FK, et al. Threonine-120 phosphorylation regulated by phosphoinositide-3-kinase/Akt and mammalian target of rapamycin pathway signaling limits the antitumor activity of mammalian sterile 20-like kinase 1.J Biol Chem. 2012 Jul 6;287(28):23698-709. E [3]. Haagensen EJ, et al. The synergistic interaction of MEK and PI3K inhibitors is modulated by mTOR inhibition. Br J Cancer. 2012 Apr 10;106(8):1386-94.

实验参考：

Cell Assay	Cells are treated with KU-0063794 for 24, 48, and 72 hours, and the medium is changed every 24 hours with freshly dissolved KU-0063794. For the measurement of cell growth, cells are washed once with PBS, and fixed in 4% (v/v) paraformaldehyde in PBS for 15 minutes. After washing once with water, the cells are stained with 0.1% Crystal Violet in 10% ethanol for 20 minutes and washed three times with water. Crystal Violet is extracted from cells with 0.5 mL of 10% (v/v) ethanoic (acetic) acid for 20 minutes. The eluate is then diluted 1:10 in water and absorbance at 590 nm is quantified. For the assessment of cell cycle distribution, cells are harvested by trypsinization, washed once in PBS, and re-suspended in ice-cold aq. 70% (v/v) ethanol. Cells are washed twice in PBS plus 1% (w/v) BSA and stained for 20 minutes in PBS plus 0.1% (v/v) Triton X-100 containing 50 g/mL propidium iodide and 50 g/mL RNase A. The DNA content of cells is determined using a FACSCalibur flow cytometer and CellQuest software. Red fluorescence (585 nm) is acquired on a linear scale, and pulse width analysis is used to exclude doublets. Cell-cycle distribution is determined using FlowJo software. [1]
Kinase Assay	HEK-293 cells are freshly lysed in Hepes lysis buffer. Lysate (1-4 mg) is pre-cleared by incubating with 5-20 μL of Protein G-Sepharose conjugated to pre-immune IgG. The lysate extracts are then incubated with 5-20 μL of Protein G-Sepharose conjugated to 5-20 μg of either anti-Rictor or anti-Raptor antibody, or pre-immune IgG. All antibodies are covalently conjugated to Protein G-Sepharose. Immunoprecipitations are carried out for 1 hour at 4°C on a vibrating platform. The immunoprecipitates are washed four times with Hepes lysis buffer, followed by two washes with Hepes kinase buffer. For Raptor immunoprecipitates used for phosphorylating S6K1, for the initial two wash steps the buffer includes 0.5mol/LNaCl to ensure optimal kinase activity. GST-Akt1 is isolated from serum-deprived HEK-293 cells incubated with PI-103 (1 μM for 1 hour). GST-S6K1 is purified from serum-deprived HEK-293 cells incubated with rapamycin (0.1 μM for 1 hour). mTOR reactions are initiated by adding 0.1 mM ATP and 10 mM $MgCl_2$in the presence of various concentrations of KU-0063794 and GST-Akt1 (0.5 μg) or GST-S6K1 (0.5 μg). Reaction are carried

	out for 30 minutes at 30°C on a vibrating platform and stopped by addition of SDS sample buffer. Reaction mixtures are then filtered through a 0.22-μM-poresize Spin-X filter and samples are subjected to electrophoresis and immunoblot analysis with the indicated antibodies. [1]
References	[1]. Garcia-Martinez et al. Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). Biochem.J. (2009)421 29. [2]. Collak FK, et al. Threonine-120 phosphorylation regulated by phosphoinositide-3-kinase/Akt and mammalian target of rapamycin pathway signaling limits the antitumor activity of mammalian sterile 20-like kinase 1.J Biol Chem. 2012 Jul 6;287(28):23698-709. E [3]. Haagensen EJ, et al. The synergistic interaction of MEK and PI3K inhibitors is modulated by mTOR inhibition. Br J Cancer. 2012 Apr 10;106(8):1386-94.