

产品名称: **PLX-4720**

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生物活性:						
Description	PLX-4720 is a potent and selective inhibitor of B-Raf ^{V600E} with IC ₅₀ of 13 nM in a cell-free assay, equally potent to c-Raf-1(Y340D and Y341D mutations), and 10-fold selectivity for B-Raf ^{V600E} than wild-type B-Raf.					
IC ₅₀ & Target	B-Raf ^{V600E}	B-Raf	BRK	FRK	Csk	Src
	13 nM (IC ₅₀)	160 nM (IC ₅₀)	130 nM (IC ₅₀)	1300 nM (IC ₅₀)	1500 nM (IC ₅₀)	1700 nM (IC ₅₀)
	FAK	FGFR	KDR	HGK	CSF1R	Aurora A
	1700 nM (IC ₅₀)	1900 nM (IC ₅₀)	2300 nM (IC ₅₀)	2800 nM (IC ₅₀)	3300 nM (IC ₅₀)	3400 nM (IC ₅₀)
In Vitro	PLX-4720 displays >10 times selectivity against wild type B-Raf, and >100 times selectivity over other kinases such as Frk, Src, Fak, FGFR, and Aurora A with IC ₅₀ of 1.3-3.4 μM. PLX-4720 significantly inhibits the ERK phosphorylation in cell lines bearing B-Raf ^{V600E} with IC ₅₀ of 14-46 nM, but not the cells with wild-type B-Raf. PLX-4720 significantly inhibits the growth of tumor cell lines bearing the B-Raf ^{V600E} oncogene, such as COLO205, A375, WM2664, and COLO829 with GI ₅₀ of 0.31 μM, 0.50 μM, 1.5 μM, and 1.7 μM, respectively. In addition, PLX-4720 treatment at 1 μM induces cell cycle arrest and apoptosis exclusively in the B-Raf ^{V600E} -positive 1205Lu cells, but not in the B-Raf wild-type C8161 cells[1].PLX-4720 treatment (10 μM) significantly induces > 14-fold expression of BIM in the PTEN+ cells, compared with the PTEN- cell lines (4-fold), giving an explanation of the resistance of PTEN- cells to PLX-4720-induced apoptosis[2].					
In Vivo	Oral administration of PLX-4720 at 20 mg/kg/day induces significant tumor growth delays and regressions in B-Raf ^{V600E} -dependent COLO205 tumor xenografts, without obvious adverse effects in mice even at dose of 1 g/kg. PLX-4720 at 100 mg/kg twice daily almost completely eliminates the 1205Lu xenografts bearing B-Raf ^{V600E} , while has no activity against C8161 xenografts bearing wild-type B-Raf. The anti-tumor effects of PLX-4720 correlate with the blockade of MAPK pathway in those cells harboring the V600E mutation[1]. PLX-4720 treatment at 30 mg/kg/day significant inhibits the tumor growth of 8505c xenografts by >90%, and dramatically decreases distant lung metastases[3].					
Solvent&Solubility	In Vitro: DMSO : ≥ 50 mg/mL (120.82 mM) * "≥" means soluble, but saturation unknown.					
	<div>Preparing Stock Solutions</div>	<div>Solvent / Mass / Concentration</div>	1 mg	5 mg	10 mg	
		1 mM	2.4165 mL	12.0823 mL	24.1645 mL	
		5 mM	0.4833 mL	2.4165 mL	4.8329 mL	
		10 mM	0.2416 mL	1.2082 mL	2.4165 mL	
<p>*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。</p> <p>储备液的保存方式和期限 -80℃, 6 months; -20℃, 1 month。 -80℃ 储存时，请在 6 个月内使用，-20℃ 储存时，请在 1 个月内使用。</p> <p>In Vivo:</p> <p>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：</p> <p>——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</p>						

	<p>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</p> <p>1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline Solubility: ≥ 5 mg/mL (12.08 mM); Clear solution</p> <p>此方案可获得 ≥ 5 mg/mL (12.08 mM, 饱和度未知) 的澄清溶液。</p> <p>以 1 mL 工作液为例，取 100 μL 50.0 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。</p> <p>2.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 5 mg/mL (12.08 mM); Clear solution</p> <p>此方案可获得 ≥ 5 mg/mL (12.08 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。</p> <p>以 1 mL 工作液为例，取 100 μL 50.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。</p>
References	<p>[1]. Tsai J, et al. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. <i>Proc Natl Acad Sci U S A</i>, 2008, 105(8), 3041-3046.</p> <p>[2]. Paraiso KH, et al. PTEN loss confers BRAF inhibitor resistance to melanoma cells through the suppression of BIM expression. <i>Cancer Res</i>, 2011, 71(7), 2750-2760.</p> <p>[3]. Nucera C, et al. B-Raf(V600E) and thrombospondin-1 promote thyroid cancer progression. <i>Proc Natl Acad Sci U S A</i>, 2010, 107(23), 10649-10654.</p> <p>[4]. Rizzolio S, et al. Neuropilin-1 upregulation elicits adaptive resistance to oncogene-targeted therapies. <i>J Clin Invest</i>. 2018 Aug 31;128(9):3976-3990.</p>
实验参考：	
Cell Assay	<p>Cells are treated with various concentrations PLX-4720 for 24, 48, and 72 hours. Cell proliferation is measured by using the CellTiter-Glo Luminescent Cell Viability Assay or MTT assay. For cell cycle analysis, supernatant and cells are collected, pelleted, and fixed with 70% ethanol. Before staining with propidium iodide (10 μg/mL), cells are incubated for 1 hour at 37°C in 0.5 mg/mL RNase I to rid samples of residual RNA contamination. Samples are then analyzed by using the EPICS XL apparatus. For the assessment of apoptosis, media and cells are harvested and pelleted before staining with annexin-FITC and propidium iodide. Samples are subsequently analyzed by using the EPICS XL apparatus. [1]</p>
Animal Administration	<p>Metastatic melanoma cells (2×10^6) are s.c. injected into the flanks of SCID mice and allowed appr 2 weeks to reach 0.125 mm³ in volume. Subsequently, the animals receive either 100 mg/kg PLX4720 (oral gavage) or vehicle control twice daily for 15 days. Tumor volume is recorded every 72 h. The average tumor size for each respective group is normalized to the tumor volume at the first day of treatment. After 15 days of treatment, animals are killed and tumors are excised, fixed in formalin, paraffin-embedded, and analyzed by immunohistochemistry. [1]</p>
Kinase Assay	<p>For each enzyme (0.1 ng), 20-μL reactions are carried out in 20 mM Hepes (pH 7.0), 10 mM MgCl₂, 1 mM DTT, 0.01% Tween-20, 100 nM biotin-MEK protein, various ATP concentrations, and increasing concentrations of PLX-4720 at room temperature. Reactions are stopped at 2, 5, 8, 10, 20, and 30 minutes with 5 μL of a solution containing 20 mM Hepes (pH 7.0), 200 mM NaCl, 80 mM EDTA, and 0.3% BSA. The stop solution also includes phospho-MEK Antibody, Streptavidin-coated Donor beads and Protein A Acceptor beads from the AlphaScreen Protein A Detection Kit. The</p>

	antibody and beads are preincubated in stop solution in the dark at room temperature for 30 minutes. The final dilution of antibody is 1/2,000, and the final concentration of each bead is 10 µg/mL. The assay plates are incubated at room temperature for one hour then are read on a PerkinElmer AlphaQuest reader. [1]
References	<p>[1]. Tsai J, et al. <u>Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity</u>. Proc Natl Acad Sci U S A, 2008, 105(8), 3041-3046.</p> <p>[2]. Paraiso KH, et al. <u>PTEN loss confers BRAF inhibitor resistance to melanoma cells through the suppression of BIM expression</u>. Cancer Res, 2011, 71(7), 2750-2760.</p> <p>[3]. Nucera C, et al. <u>B-Raf(V600E) and thrombospondin-1 promote thyroid cancer progression</u>. Proc Natl Acad Sci U S A, 2010, 107(23), 10649-10654.</p> <p>[4]. Rizzolio S, et al. <u>Neuropilin-1 upregulation elicits adaptive resistance to oncogene-targeted therapies</u>. J Clin Invest. 2018 Aug 31;128(9):3976-3990.</p>



源叶生物