产品名称：**IPA-3**
产品别名：**IPA-3**

生物活性：

Description	IPA-3 is a selective non-ATP competitive PAK1 inhibitor with IC_{50} of 2.5 μM, and shows no inhibition to group II PAKs (PAKs 4-6).
IC_{50} & Target	PAK1
	2.5 μM (IC_{50})
In Vitro	IPA-3 inhibits Pak1 activation in part by binding covalently to the regulatory domain of Pak1. IPA-3 binds Pak1 covalently in a time- and temperature-dependent manner. IPA-3 prevents binding of the Pak1 activator Cdc42. IPA-3 binds directly to the Pak1 autoregulatory domain. IPA-3 reversibly inhibits PMA-induced membrane ruffling in cells[1]. IPA-3 (2 μM, 5 μM or 20 μM) reduces cell spreading in human primary Schwann and schwannoma cells. IPA-3 treatment significantly reduces the number of adherent Schwann and schwannoma cells in a dose-dependent manner[2]. IPA-3 is a non ATP-competitive, allosteric inhibitor of p21-activated kinase 1 (Pak1). PIR3.5 is the control compound of IPA-3. IPA-3 prevents Cdc42-stimulated Pak1 autophosphorylation on Thr423. IPA-3 also prevents sphingosine-dependent Pak1 autophosphorylation. IPA-3 does not target exposed cysteine residues on Pak1. The disulfide bond of IPA-3 is critical for inhibition of Pak1 and in vitro reduction by the reducing agent dithiothreitol (DTT) abolishes Pak1 inhibition by IPA-3. IPA-3 inhibits activation of Pak1 by diverse activators, but does not inhibit preactivated Pak1. IPA-3 inhibits PDGF-stimulated Pak activation in mouse embryonic fibroblasts[3].
Solvent&Solubility	***In Vitro:***
	DMSO : ≥ 100 mg/mL (285.35 mM)
	H_2O : < 0.1 mg/mL (insoluble)
	* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.8535 mL	14.2674 mL	28.5347 mL
	5 mM	0.5707 mL	2.8535 mL	5.7069 mL
	10 mM	0.2853 mL	1.4267 mL	2.8535 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: ≥ 2.5 mg/mL (7.13 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (7.13 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 25.0 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。 2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: 2.5 mg/mL (7.13 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (7.13 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 25.0 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: ≥ 2.5 mg/mL (7.13 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (7.13 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 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Viaud J, et al. An allosteric kinase inhibitor binds the p21-activated kinase autoregulatory domain covalently. Mol Cancer Ther. 2009 Sep;8(9):2559-65. [2]. Flaiz C, et al. PAK kinase regulates Rac GTPase and is a potential target in human schwannomas. Exp Neurol. 2009 Jul;218(1):137-44. [3]. Deacon SW, et al. An isoform-selective, small-molecule inhibitor targets the autoregulatory mechanism of p21-activated kinase. Chem Biol. 2008 Apr;15(4):322-31.

实验参考：

Cell Assay	Human primary schwannoma cells are grown on 96 well plates for 2 days. Cells are left untreated or treated with 5 μM IPA-3, 20 μM IPA-3 or 20 μM PIR-3.5 for 24 hours. The MTS-solution is left on the cells for 3 hours, before the absorbance at 490 nm is measured. The experiments are conducted three times and mean and standard error of the mean is calculated with Excel. [2]
Kinase Assay	Pak1 (150 nM final) is pre-incubated with MBP (8.3 μM), indicated proteins, and IPA-3 or DMSO in Kinase buffer for 20 minutes at 4°C. Cdc42-GTPγS (3.2 μM) is then added and the reaction is pre-equilibrated 10 minutes at 30°C. Kinase reactions are started by the addition of ATP (to 30 μM) containing [^{32}P]ATP and are incubated 10 min and analyzed by SDS-PAGE and autoradiography. [1]
References	[1]. Viaud J, et al. An allosteric kinase inhibitor binds the p21-activated kinase autoregulatory domain covalently. Mol Cancer Ther. 2009 Sep;8(9):2559-65. [2]. Flaiz C, et al. PAK kinase regulates Rac GTPase and is a potential target in human schwannomas. Exp Neurol. 2009 Jul;218(1):137-44. [3]. Deacon SW, et al. An isoform-selective, small-molecule inhibitor targets the autoregulatory mechanism of p21-activated kinase. Chem Biol. 2008 Apr;15(4):322-31.

源叶生物