产品名称：**K-115**
产品别名：**Ripasudil**

生物活性：

Description	Ripasudil (K-115) is a specific inhibitor of ROCK, with IC_{50}s of 19 and 51 nM for ROCK2 and ROCK1, respectively.				
IC_{50} & Target	ROCK2	ROCK1	CaMKIIa	PKACa	PKC
	19 nM (IC_{50})	51 nM (IC_{50})	370 nM (IC_{50})	2.1 μM (IC_{50})	27 μM (IC_{50})
In Vitro	Ripasudil (K-115) is a potent inhibitor of ROCK, with IC_{50}s of 19 and 51 nM for ROCK2 and ROCK1, respectively. Ripasudil also shows less potent inhibitory activities against CaMKIIα, PKACα and PKC, with IC_{50}s of 370 nM, 2.1 μM and 27 μM, respectively [1]. Ripasudil (K-115; 1, 10 μM) induces cytoskeletal changes, including retraction and cell rounding and reduced actin bundles of cultured trabecular meshwork (TM) cells. Ripasudil (5 μM) sifnificantly reduces transendothelial electrical resistance (TEER), and increases FITC-dextran permeability in Schlemm's canal endothelial (SCE) cell monolayers [2].				
In Vivo	Ripasudil (K-115) reduces intraocular pressure (IOP) in a concentration-dependent manner at concentrations between 0.1% and 0.4% in monkey eyes and 0.0625% to 0.5% in rabbit eyes, respectively[1]. Ripasudil (K-115; 1 mg/kg, p.o. daily) shows a neuroprotective effect on retinal ganglion cells (RGCs) after nerve crush (NC). Ripasudil also inhibits the oxidative stress induced by axonal injury in mice. Ripasudil suppresses the time-dependent production of ROS in RGCs after NC injury[3].				
Solvent&Solubility	***In Vitro:*** **H_2O : ≥ 50 mg/mL (126.30 mM)** * "≥" means soluble, but saturation unknown.				
	Preparing Stock Solutions	**Solvent / Mass / Concentration**	**1 mg**	**5 mg**	**10 mg**
		1 mM	2.5260 mL	12.6301 mL	25.2602 mL
		5 mM	0.5052 mL	2.5260 mL	5.0520 mL
		10 mM	0.2526 mL	1.2630 mL	2.5260 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。				
References	[1]. Isobe T, et al. Effects of K-115, a rho-kinase inhibitor, on aqueous humor dynamics in rabbits. Curr Eye Res. 2014 Aug;39(8):813-22. [2]. Kaneko Y, et al. Effects of K-115 (Ripasudil), a novel ROCK inhibitor, on trabecular meshwork and Schlemm's canal endothelial cells. Sci Rep. 2016 Jan 19;6:19640. [3]. Yamamoto K, et al. The novel Rho kinase (ROCK) inhibitor K-115: a new candidate drug for neuroprotective treatment in glaucoma. Invest Ophthalmol Vis Sci. 2014 Oct 2;55(11):7126-36.				

实验参考：

	Trabecular meshwork (TM) cells are plated on 6 well plates at a density of 1×10^4 cells per well in DMEM containing 10% FBS. Following overnight culture, when cells have reached semiconfluence, 1 or 10 μM of Ripasudil, 10 μM of Y-27632, or 10 μM of fasudil are added to culture wells. PBS is used as a control vehicle. After 60 min, drug solutions are removed and replaced with

Cell Assay	DMEM containing 10% FBS. Cells are observed by phase-contrast microscopy and photographed 60 min after drug application and 2 h after drug removal. For immunohistochemistry, TM cells are plated on gelatin-coated 8 well chamber slides at a density of 1×10^4 cells per well in DMEM containing 10% FBS. After overnight culture, when cells reach semiconfluence, cell are incubated in Ripasudil at 1 or 10 μM, Y-27632 at 10 μM, or fasudil at 10 μM for 60 min. PBS is used as a control vehicle. Drug solutions are removed and replaced with DMEM containing 10% FBS after 2 h. Cells are fixed with 4% paraformaldehyde in PBS for 15 min then washed with cytoskeletal buffer (10 mM MES, 150 mM NaCl, 5 mM EGTA, 5 mM $MgCl_2$, 5 mM glucose, pH 6.1) and serum buffer (10% FBS in PBS). Cells are permeabilized with 0.5% Triton X-100 in PBS for 12 min at room temperature and blocked with serum buffer for at least 2 h at 4°C. Filamentous actin (F-actin) is labeled with 0.05 mg/mL Phalloidin-TRITC for 1 h at room temperature. After washing with PBS, cells are mounted with commercial mounting medium containing DAPI and observed using a fluorescence microscope. The exposure to take images for F-actin and DAPI are 0.1 and 0.05 sec, respectively [2]
Animal Administration	Rabbits [1] In the rabbit experiments, 50 mL of vehicle or Ripasudil at concentrations of 0.0625%, 0.125%, 0.25, or 0.5% is instilled into one eye. Intraocular pressure (IOP) is measured in both eyes before and 0.5, 1, 2, 3, 4, and 5 h after instillation. The contralateral eye is not treated. Animals are administered all concentrations of Ripasudil assigned using the Latin square method with intervals of at least 2 d. Monkeys [1] In the monkey experiments, 20 mL of Ripasudil at concentrations of 0.1%, 0.2%, or 0.4%, and latanoprost at a concentration of 0.005% are instilled into one eye. IOP is measured in both eyes before and 1, 2, 4, 6, and 8 h after instillation. The contralateral eye is not treated. Animals are arranged to receive all formulations with intervals of at least 1 week using the Latin square method. The IOPs are compared with the results for the instillation side at pre-dose and at each time point after instillation of Ripasudil, and are compared with both eyes at each time point.
Kinase Assay	ROCK 1 (0.75 ng/mL) and ROCK 2 (0.5 ng/mL) are incubated with various concentrations of Ripasudil, Y-27632, or HA-1077 at 25°C for 90 min in 50 mM Tris-HCl buffer (pH 7.5) containing 100 mM KCl, 10 mM $MgCl_2$, 0.1 mM EGTA, 30 mM Long S6 Kinase Substrate peptide, and 1 mM ATP in a total volume of 40 mL. PKACa, PKC, and CaMKIIa are also incubated with various concentrations of Ripasudil, Y-27632, or HA-1077. PKACa (0.0625 ng/mL) is incubated at 25°C for 30 min in 40 mM Tris-HCl buffer (pH 7.5) containing 20 mM $MgCl_2$, 1 mg/ mL BSA, 5 mM Kemptide peptide substrate, and 1 mM ATP in a total volume of 40 mL. PKC (0.025 ng/mL) is incubated at 25°C for 80 min in 20 mM Tris-HCl buffer (pH 7.5) containing 20 mM $MgCl_2$, 0.4 mM $CaCl_2$, 0.1 mg/mL BSA, 0.25 mM EGTA, 25 ng/mL phosphatidylserine, 2.5 ng/mL diacylglycerol, 0.0075% Triton-X-100, 25 mM DTT, 10 mM Neurogranin (28-43) peptide substrate, and 1 mM ATP in a total volume of 40 mL. CaMKIIa (0.025 ng/mL) is incubated at 25°C for 90 min in 50 mM Tris-HCl buffer (pH 7.5) containing 10 mM $MgCl_2$, 2 mM $CaCl_2$, 0.04 mg/mL BSA, 16 mg/mL purified calmodulin from bovine testis, 500 mM DTT, 50 mM Autocamitide 2, and 1 mM ATP in a total volume of 40 mL. After incubation, 40 mL of KinaseGlo Luminescent Kinase Assay solution is added, and allowed to remain at 25°C for 10 min, and Relative Light Units (RLU) are measured using a luminometer. The RLU without test compound is set as 100% (Control value), and that without enzyme and compound is set as 0% (Normal value). The reaction rate (% of control) is then calculated from the RLU with

	addition of each concentration of test compounds, and the 50% inhibitory concentrations (IC_{50}) are determined by logistic regression analysis using SAS [1]
References	[1]. Isobe T, et al. Effects of K-115, a rho-kinase inhibitor, on aqueous humor dynamics in rabbits. Curr Eye Res. 2014 Aug;39(8):813-22. [2]. Kaneko Y, et al. Effects of K-115 (Ripasudil), a novel ROCK inhibitor, on trabecular meshwork and Schlemm's canal endothelial cells. Sci Rep. 2016 Jan 19;6:19640. [3]. Yamamoto K, et al. The novel Rho kinase (ROCK) inhibitor K-115: a new candidate drug for neuroprotective treatment in glaucoma. Invest Ophthalmol Vis Sci. 2014 Oct 2;55(11):7126-36.