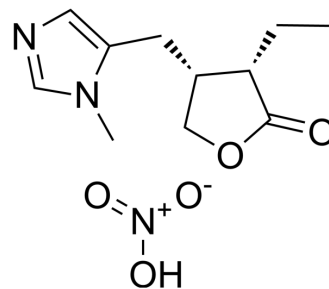


## Pilocarpine nitrate

Cat. No.:	HY-B1006
CAS No.:	148-72-1
Molecular Formula:	C <sub>11</sub> H <sub>17</sub> N <sub>3</sub> O <sub>5</sub>
Molecular Weight:	271.27
Target:	mAChR
Pathway:	GPCR/G Protein; Neuronal Signaling
Storage:	4°C, sealed storage, away from moisture * The compound is unstable in solutions, freshly prepared is recommended.



### SOLVENT & SOLUBILITY

In Vitro	DMSO : 250 mg/mL (921.59 mM; Need ultrasonic)					
	H <sub>2</sub> O : 100 mg/mL (368.64 mM; Need ultrasonic)					
	Preparing Stock Solutions	Solvent Concentration	Mass	1 mg	5 mg	10 mg
			1 mM	3.6864 mL	18.4318 mL	36.8636 mL
			5 mM	0.7373 mL	3.6864 mL	7.3727 mL
10 mM			0.3686 mL	1.8432 mL	3.6864 mL	
Please refer to the solubility information to select the appropriate solvent.						
In Vivo	1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.08 mg/mL (7.67 mM); Clear solution					
	2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.08 mg/mL (7.67 mM); Clear solution					
	3. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.08 mg/mL (7.67 mM); Clear solution					

### BIOLOGICAL ACTIVITY

Description	Pilocarpine nitrate is a potent M3-type muscarinic acetylcholine receptor (M3 muscarinic receptor) agonist.
IC <sub>50</sub> & Target	mAChR3
In Vitro	To evaluate the cytotoxicity of Pilocarpine, the morphology and viability of human corneal stromal (HCS) cells are examined by light microscopy and MTT assay, respectively. Morphological observations show that HCS cells exposed to Pilocarpine at a concentration from 0.625 to 20 g/L exhibit dose- and time-dependent proliferation retardation and morphological abnormality such as cellular shrinkage, cytoplasmic vacuolation, detachment from culture matrix, and eventually death,

while no obvious difference is observed between those exposed to Pilocarpine below the concentration of 0.625 g/L and controls. Results of MTT assay reveal that the cell viability of HCS cells decrease with time and concentration after exposing to Pilocarpine above the concentration of 0.625 g/L ( $P < 0.01$  or  $0.05$ ), while that of HCS cells treated with Pilocarpine below the concentration of 0.625 g/L show no significant difference to controls<sup>[2]</sup>. The partial muscarinic agonist, Pilocarpine, evokes concentration-dependent relaxation with an  $EC_{50}$  of 2.4 mM in isolated segments of rat tail artery that were constricted with Penylephrine (10 to 200 nM)<sup>[3]</sup>.

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

#### In Vivo

The Pilocarpine-induced saliva secretion of the control rats (CN) and exercised (EX) rats is examined. A significantly greater amount of saliva is induced by Pilocarpine in the EX rats than in the CN rats ( $P < 0.01$ ). Conversely, the  $Na^+$  concentration in the saliva of the EX rats is significantly lower than that of the CN rats ( $P < 0.05$ )<sup>[1]</sup>.

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

## PROTOCOL

### Cell Assay <sup>[2]</sup>

Cell viability is determined by MTT assay. Briefly, HCS cells are inoculated into a 96-well culture plate (Nunc) at a density of  $1 \times 10^4$  cells/100  $\mu$ L/well, and are cultured and treated. At a 4h interval, the Pilocarpine (0.625 to 20 g/L)-containing medium is replaced entirely with 100  $\mu$ L serum-free DMEM/F12 medium containing 1.0 g/L MTT, and the cells are incubated at 37°C in the dark for 4h. After the MTT-containing medium is discarded with caution, 150  $\mu$ L DMSO is added to dissolve the produced formazan crystals at 37°C in the dark for 15 min, and the absorbance at 490 nm is measured with a Multiskan GO microplate reader<sup>[2]</sup>.

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

### Animal Administration <sup>[1]</sup>

Rats<sup>[1]</sup>

Male, 10-week-old Wistar rats are assigned to one of two groups, exercise (EX,  $n=6$ ) and control (CN,  $n=6$ ). The EX rats are kept for 40 days in cages with a running wheel (SN-451), allowing them to undertake voluntary exercise, while the CN rats are kept in cages with the running wheel locked. On the 40th day, Pilocarpine-induced saliva is measured as follows. Briefly, the rats are anesthetized, preweighed cotton was placed in their mouths sublingually, and Pilocarpine (0.5 mg/kg) is intraperitoneally injected to induce saliva secretion. Each cotton ball is then changed every 10 min for 1 h. The collected cotton balls are weighed again, and the mass of saliva secreted is calculated by subtracting the initial from the final weight.

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

## CUSTOMER VALIDATION

- Immunity. 2022 Jul 12;S1074-7613(22)00291-6.
- Cell Rep. 2023 Apr 30;42(5):112456.
- Food Chem. 30 November 2022, 133593.
- Prog Neurobiol. 2022 Aug 2;102335.
- Life Sci. 2022 Nov 1;308:120942.

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## REFERENCES

[1]. Matsuzaki K, et al. Daily voluntary exercise enhances pilocarpine-induced saliva secretion and aquaporin 1 expression in rat submandibular glands. FEBS Open Bio. 2017 Dec 7;8(1):85-93.

[2]. Yuan XL, et al. Cytotoxicity of pilocarpine to human corneal stromal cells and its underlying cytotoxic mechanisms. Int J Ophthalmol. 2016 Apr 18;9(4):505-11.

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[3]. Tonta MA, et al. Pilocarpine-induced relaxation of rat tail artery by a non-cholinergic mechanism and in the absence of an intact endothelium. Br J Pharmacol. 1994 Jun;112(2):525-32.

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**Caution: Product has not been fully validated for medical applications. For research use only.**

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