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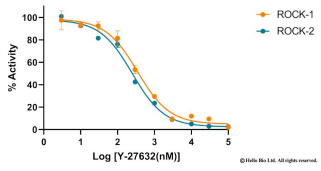
DATASHEET

ROCK inhibitor Y-27632

Product overview

Name	ROCK inhibitor Y-27632
Cat No	HB2297
Alternative names	Y27632 dihydrochloride
Biological action	Inhibitor
Purity	>99%
Customer comments	*The ROCK inhibitor Y-27632 is working as expected.* **Verified customer, University of Louisville** *Worked well just as it should do. Kept me informed of when product would be delivered and notified me when delivered. Good packaging and data sheet. Easy to dissolve and use and it worked!* **Verified customer, University of Cardiff**
Description	Y-27632 is a selective ROCK inhibitor. Frequently used 3D growth matrix component and for production of organoids. Also used when inducing neurons from fibroblasts.

Images



Biological Data

Biological description

The ROCK inhibitor Y-27632 is a selective and cell permeable inhibitor of the Rho-associated protein kinase (ROCK) (K_i values are 0.14, 26 ,25 and >250 µM at p160ROCK, PKC, PKA and MLCK respectively).

Y-27632 has many biological actions and is an important small molecule modulator of stem cells. It diminishes hESC and hiPSC dissociation-induced apoptosis, enhances survival and colony formation of dissociated hESC without affecting pluripotency or self-renewal. Y-27632 also improves hESC and iPSCs postthaw viability and survival rate during cryopreservation. It is frequently used as a 3D growth matrix component and for production of organoids (e.g. brain organoids). Additionally, Y-27632 inhibits smooth muscle contractility and shows antihypertensive effect.

Background

Rho kinase (Rho-associated coiled coil protein kinase or ROCK) is a major downstream effector for the small GTP-binding protein Rho [1]. Rho kinase is involved in various biological processes including cell mitosis adhesion, cytoskeletal adjustments, muscle cell contraction, tumor cell invasion and a series of cell biological phenomena [2]. Through a series of cascading effects, Rho kinase influences actin and microtubule regulation of apoptosis and thus plays a key role in early embryonic development [3].The

Rho kinase signalling pathway is highly activated in many pathological conditions [4].

Rock inhibitor Y-27632 is a Rho kinase inhibitor which selectively inhibits p160ROCK (ROCK1) with a K_i value of 140 nM and binds to the Rho kinase ATP binding pocket in an ATP-competitive manner [5].

Uses

Y-27632 has been shown to have a diverse range of actions and has been used in a variety of biological systems. It is the most commonly used of the ROCK inhibitors in stem cell research and is used as a multifunctional reagent in many stem cell related processes [6]. Y-27632 demonstrates many positive effects across various cell types, in particular human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) [7] and has greatly improved a number of practical stem cell procedures [8]. For example it has been shown that Y-27632 increases cell adhesion and proliferation and increases post-thaw cell survival and post-passaging cell viability [9,10].

Cellular dissociation

Cell dissociation is one of the most common manipulations in stem cell research. During cellular dissociation both hESC and hIPSCs are vulnerable to dissociation-induced apoptosis or anoikis (a form of apoptosis induced by inappropriate cell-cell or cell-ECM interactions) [3,11]. Y-27632 is of particular interest as it allows hPSCs to escape this dissociation-induced apoptosis. Y-27632 prevents apoptosis of dissociated hPSCs and increases their survival rate and plating efficiency [11].

Cryopreservation

Cryopreservation is also an important part of stem cell research. Y-27632 improves the recovery of cryopreserved hPSCs and has greatly helped simplify the cryopreservation procedure. The slow-freezing and rapid-thawing procedure which uses DMSO as a cryoprotectant is commonly used in stem cell cryopreservation, however this method is not suitable for hPSCs, therefore vitrification (fast freezing, slow thawing) is used [12]. Vitrified hPSCs do however still suffer from high levels of cell death and do not passage well as single cells [11]. The addition of Y-27632 to freezing and post-thawing medium enhances colony formation efficiency and treatment with Y-27632 during cryopreservation also increases survival rate and cell adhesion of freeze-thawed dissociated hES [12].

Application notes

#Protocol 1: ROCK Inhibition assay using Y-27632

- ROCK-I and ROCK-II were assayed against Long S6 substrate peptide in a buffer containing 0.1mM ^{33}P-g-ATP.
- Enzymes were incubated with substrate for 30 minutes at room temperature before the reaction was stopped with 0.5M orthophosphoric acid.
- Samples were transferred to a P81 unifilter plate before being washed and then remaining ^{33}P labelled substrate being detected with a scintillation counter.

Solubility & Handling

Storage instructions
Room temperature (desiccate)

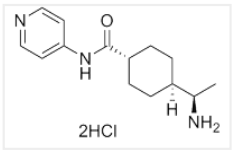
Solubility overview
Soluble in water (100mM), PBS (100mM), DMSO (100mM)

Handling

- If using the compound as a cell culture supplement, you should dilute the stock solution into culture medium immediately before use.
- Cell culture media can be prewarmed before adding the compound to avoid potential precipitation of the compound.
- Y-2762 is often removed from medium within 20 hours
- The compound has been shown to be effective at a final concentration of 10 µM (Ungrin et al.; Watanabe et al.)
- Mix and filter supplemented media using a 0.2 µm low-protein binding filter.
- Please avoid a final DMSO concentration above 0.1% due to potential cell toxicity

Important
This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use.

Chemical Data

Chemical name	*trans*-4-[(1*R*)-1-Aminoethyl]-*N*-4-pyridinylcyclohexanecarboxamide dihydrochloride
Molecular Weight	320.26
Chemical structure	
Molecular Formula	$C_{14}H_{21}N_3O.2HCl$
CAS Number	129830-38-2
PubChem identifier	9901617
SMILES	C[C@H](C1CCC(CC1)C(=O)NC2=CC=NC=C2)N.Cl.Cl
Source	Synthetic
InChi	InChI=1S/C14H21N3O.2ClH/c1-10(15)11-2-4-12(5-3-11)14(18)17-13-6-8-16-9-7-13;;/h6-12H,2-5,15H2,1H3,(H,16,17,18);2*1H/t10-,11?,12?;;/m1../s1
InChiKey	IDDDVXIUIXWAGJ-DDSAHXNVSA-N
MDL number	MFCD03490488
Appearance	White solid

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