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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 Works just as intended, at half the price of what it costs at another company. Verified customer,

University of Alabama at Birmingham, USA

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

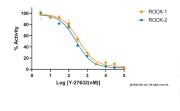
worked! Verified customer, University of Cardiff, UK

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. Manufactured to a high

degree of optical purity.

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 μ M 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

- \bullet ROCK-I and ROCK-II were assayed against Long S6 substrate peptide in a buffer containing 0.1mM $^{33}\text{P-g-ATP}.$
- 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 ³³P labelled substrate being detected with a scintillation counter.

Solubility & Handling

Solubility overview Storage instructions Storage of solutions Soluble in water (100mM), PBS (100mM), DMSO (100mM) Room temperature (desiccate)

Prepare and use solutions on the same day if possible. Store solutions at -20 °C for up to one month if storage is required. Equilibrate to RT and ensure the solution is precipitate free before use.

- 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
- \bullet The compound has been shown to be effective at a working concentration of 10 μM (Ungrin et al.; Watanabe et al.)
- \bullet 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

Handling

Shipping Conditions

Stable for ambient temperature shipping. Follow storage instructions on receipt.

Chemical Data

Chemical name trans-4-[(1R)-1-Aminoethyl]-N-4-pyridinylcyclohexanecarboxamide dihydrochloride

Molecular Weight 320.26

Chemical structure 2HCI

Molecular Formula C₁₄H₂₁N₃O.2HCl **CAS Number** 129830-38-2 **PubChem identifier** 9901617

SMILES C[C@H](C1CCC(CC1)C(=O)NC2=CC=NC=C2)N.CI.CI

Source Synthetic

InChi InChI=1S/C14H21N3O.2CIH/c1-10(15)11-2-4-12(5-3-11)14(18)17-13-6-8-16-9-7-13;;/h6-12H,2-5,15

H2,1H3,(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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