Product Sheet

ROCK Inhibitor Y27632 (ATCC® ACS-3030™)

Please read this FIRST

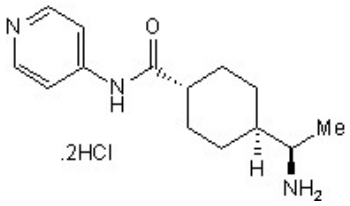
Storage Temp.
-20°C or colder

Biosafety Level
*

Description

Product Description:
ROCK Inhibitor Y27632 is a selective inhibitor of the Rho-associated kinase p160ROCK. Treatment with ROCK Inhibitor Y27632 prevents dissociation-induced apoptosis of human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC), increasing the survival rate and maintaining pluripotency during subcultivation and thawing of hESCs and hiPSCs. ROCK Inhibitor Y27632 also has been shown to enhance the survival rate of stem cells during cryopreservation.

Structure of ROCK Inhibitor Y27632



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

Volume: 10 mg

Directions for Use

Directions for Use Detailed protocols relating to this product are available online at http://www.atcc.org/Guides/Primary_Cell_Culture_Guide.aspx.

Prepare a 10 mM stock solution.

Add 3 mL of sterile water to the 10 mg vial of ROCK Inhibitor Y27632. Mix thoroughly.

Aliquot the stock solution in working volumes based on routine use. Note that ROCK Inhibitor Y27632 is used at a final concentration of 10 µM (1:1000 dilution) in cell culture medium. Store aliquots at -20°C and avoid repeated freezing and thawing. Once thawed, aliquots may be kept at 2°C to 8°C for two weeks.

Cell culture medium: Pluripotent Stem Cell SFM XF/FF (ATCC® ACS-3002) is recommended for feeder-free culture and Pluripotent Stem Cell SFM (ATCC® ACS-3001) is recommended for feeder-dependent culture (e.g., mouse embryonic fibroblasts (MEF) or human foreskin fibroblasts (HFF)).

Preparation of Medium Supplemented with ROCK Inhibitor Y27632

1. Thaw 10 mM stock solution of ROCK Inhibitor Y27632 on ice.
2. Dilute 10mM ROCK Inhibitor Y27632 1:1000 in cell culture medium to obtain a final concentration of 10 µM. *For example, if you are preparing 500 mL of media add 0.5 ml 10 mM ROCK inhibitor*
3. Medium supplemented with ROCK Inhibitor Y27632 must be used immediately.

Protocol for Passaging Cells

This protocol is designed for the passaging of cells cultured in a 6-cm dish, using Stem Cell Dissociation Reagent (ATCC® ACS-3010) to detach the cell colonies from the dish. Stem Cell Dissociation Reagent is stored as a 0.5 U/mL working solution in DMEM: F-12 Medium (ATCC[â] 30-2006).

Volumes should be adjusted according to the size and number of the tissue culture vessels to be processed.

1. Culture hESCS/hiPSCs in stem cell culture medium until cells reach 80% confluency.
2. Warm an aliquot of Stem Cell Dissociation Reagent working solution to room temperature.
3. Aspirate and discard the stem cell culture medium.
4. Rinse (add and aspirate) the cells twice with 4 mL of Dulbecco's Phosphate Buffered Saline. Remove and discard the D-PBS.
5. Add 2 mL of Stem Cell Dissociation Reagent working solution to the dish.
6. Incubate at 37°C for 10 to 15 minutes or until the edges of the individual colonies begin to loosen and fold back. View the dish under the microscope starting at 5 minutes as incubation time may vary depending on the cell line and colony size.
7. Aspirate the Stem Cell Dissociation Reagent and gently rinse (add and aspirate) the colonies by adding 4 mL of DMEM: F-12 Medium. *Take care not to dislodge the cells when aspirating the DMEM:F-12 Medium.*
8. Add 2 mL of stem cell culture medium to the dish, and detach the cells by pipetting up and down several times with a 1 mL tip. Take care not to over-pipette the culture into a single-cell suspension as single colonies will not establish colonies after seeding.
9. Transfer the cell aggregates to a 15 mL conical tube.
10. Add an additional 4 mL of stem cell culture medium to the dish to collect any remaining cells. Transfer this rinse to the 15 mL conical tube containing the cell aggregates.
11. Centrifuge the cell aggregates at 200 x g for 5 minutes.
12. Aspirate the supernatant and discard. Gently tap the bottom of the tube to loosen the cell pellet.
13. For each dish processed, add 2 mL of stem cell culture medium in the presence of 10 µM ROCK

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Or contact your local distributor

ATCC

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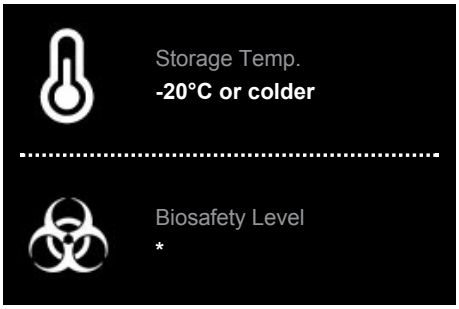

Inhibitor Y27632 to the 15-mL tube.

14. Gently resuspend the pellet by pipetting up and down **2 to 3** times with a 1 mL tip, maintaining the small cell aggregates.
15. **For feeder-free cultures**: Transfer 0.5 mL of the cell aggregates onto each CellMatrix Gel*-coated dish that contains 4 mL Complete Pluripotent Stem Cell SFM XF/FF in the presence of 10 µM ROCK Inhibitor Y27632 for a 1:4 split ratio.
    *CellMatrix™ Basement Membrane Gel (ATCC® ACS-3035)
    For feeder-dependent cultures: Transfer 0.5 mL of the cell aggregates onto each MEF or HFF-coated dish that contains 4 mL Pluripotent Stem Cell SFM XF in the presence of 10 µM ROCK Inhibitor Y27632 for a 1:4 split ratio.
16. Swiftly move the dishes in a forward to backward, then left to right pattern, once, to gently disperse the cells evenly across the surface of the dishes. Incubate dishes overnight at 37°C and 5% CO_2.
17. Change medium daily until the colonies are big enough to passage (4 to 5 days). ROCK Inhibitor Y27632 is not necessary in subsequent cell culture medium changes.

Note: The use of ROCK inhibitor may cause a transient spindle-like morphology effect on the cells. However, the colony morphology will recover after subsequent media change without ROCK inhibitor

References

1. Li X. et al, ROCK inhibitor improves survival of cryopreserved serum/feeder-free single human embryonic stem cells. Human Reproduction, Vol.24 No.3 pp 580-589, 2009 PubMed 19056770

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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