ROCK Inhibitor Y27632

ACS-3030™

Description

ROCK Inhibitor Y27632 can be used in increased primary human organoid formation efficiency and recovery from thawing and passaging. The ROCK Inhibitor Y27632 can be used in enhanced survival and maintenance of pluripotency of human embryonic stem cells (hESC), human induced pluripotent stem cells (hiPSC), and 3-D culture of organoids. Also, the inhibitor can be used in critical media components in conditional reprogramming of cells (CRC) culture technology.

Shipping information: Product provided as a 10 mg powder

Storage Conditions

Storage conditions: -20°C or colder

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

Biosafety Information

ATCC determined that a biosafety level is not applicable to this material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to complete your own risk assessment and understand any potential hazards associated with the material per your organization's policies and procedures and any other applicable regulations as enforced by your local or national

agencies.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Handling Procedures

Preparation and storage

Prepare a 10 mM stock solution

1. Aseptically add 3 mL of sterile water (ATCC 60-2450) or D-PBS (ATCC 30-2200) to the 10 mg vial of ROCK Inhibitor Y27632. Mix thoroughly by pipetting.
2. Aliquot the stock solution in working volumes based on routine use.
3. Store aliquots at -20°C to -80°C and avoid repeated freezing and thawing. Once thawed, aliquots may be kept at 2°C to 8°C for two weeks.

A. Organoid culture

- Use at a final concentration of 10 µM (1 µL of a 10 mM stock solution per mL of complete culture media).
- Supplement the media for the first 2-3 days post-thaw and during the first 2-3 days post-passaging.
- Add ROCK Inhibitor Y-27632 directly to complete culture media as needed. Do not store culture media once supplemented.

B. Conditional reprogramming of cells (CRC) culture

- Utilized as a component in F-media. Use with or without feeder cells.
- Use at a final concentration of 10 µM (1 µL of a 10 mM stock solution per mL of complete culture media)

C. Human induced pluripotent stem cells (iPSC) culture

Refer to the ATCC Stem Cell Culture Guide for more information.

Cell culture medium: Pluripotent Stem Cell SFM XF/FF (ATCC ACS-3002) is recommended for feeder free culture and ROCK Inhibitor Y-27632 is required for initial culture of iPSCs.

Preparation of Medium Supplemented with ROCK Inhibitor Y-27632

1. Thaw 10 mM stock solution of ROCK Inhibitor Y-27632 on ice.
2. Dilute 10 mM ROCK Inhibitor Y-27632 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 Y-27632 must be used immediately.

Handling Procedure for Frozen Cells and Initiation of Cultures

1. 30 Minutes Prior to Handling Cells Prewarm the appropriate stem cell culture medium at 37°C for at least 30 minutes before adding to cells.
2. Remove cryovial of frozen cells from liquid nitrogen storage.
3. Thaw the cells by gentle swirling in a 37°C water bath. To reduce the possibility of contamination, keep the Oring and cap out of the water. Thawing should be rapid (approximately 1 to 2 minutes). Remove the cryovial from the water bath when only a few ice crystals are remaining.
4. Sterilize the cryovial by rinsing with 70% ethanol.
5. Using a 1 mL or 5 mL pipette, gently transfer the cell suspension to a 15 mL conical tube.
6. Slowly add 4 mL stem cell culture medium including 10 µM of ROCK Inhibitor Y-27632 (ATCC ACS-3030) dropwise, to the conical tube. Use an additional 1 mL of media to rinse the cryovial and transfer the liquid to the 15 mL conical tube. Shake the conical tube gently to mix the cells while adding media.
7. Gently pipette the cells up and down 1-2 times to mix thoroughly. Avoid breaking apart the aggregates into a singlecell suspension.
8. Centrifuge the cells at 200 x g for 5 minutes.
9. Aspirate the supernatant and discard. Gently tap on the bottom of the tube to

loosen the cell pellet.

10. Add 1 mL of stem cell culture medium that has been supplemented with ROCK Inhibitor Y27632 (ATCC ACS-3030) to a final concentration of 10 µM. Gently resuspend the pellet by pipetting up and down 1-2 times with a 1 mL tip, maintaining the cell aggregates.
11. Plate the cells as desired under feederfree culture condition
12. ROCK Inhibitor Y-27632 is not necessary in subsequent cell culture medium changes and is not required for passaging cells.

Note: The use of ROCK inhibitor may cause a transient spindle-like morphology effect on iPSCs. 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. Hum Reprod 24(3): 580-589, 2009. PubMed: 19056770
2. Liu, Xuefeng, et al. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. Am J Pathol 180(2): 599-607, 2012.

Quality Control Specifications

Mycoplasma contamination: Not detected

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: ROCK Inhibitor Y27632 (ATCC ACS-3030)

References

References and other information relating to this material are available at www.atcc.org.

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