ATCC

Product Sheet

Stem Cell Dissociation Reagent (ATCC® ACS-3010™)

Please read this FIRST

Storage Temp.
2°C to 8°C

Biosafety Level
*

American Type Culture Collection
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Description

Product Description: Stem Cell Dissociation Reagent is a neutral protease isolated from Bacillus polymyxa that promotes safe and efficient detachment of human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC) during subcultivation in cell culture. The gentle proteolytic action of the reagent does not damage the cell membrane and supports the growth of hiPSCs in an undifferentiated state. **Serum does not inhibit the activity of the reagent.** Stem Cell Dissociation Reagent is suitable for hESCs and hiPSCs cultured in feeder-free and feeder-dependent conditions.
Volume: 250 mg

Directions for Use

Detailed protocols relating to this product are available online at www.atcc-guides.org/stemcell.

Preparation of 0.5 U/mL Working Solution

Lyophilized proteins tend to be hygroscopic. Bring the vial of Stem Cell Dissociation Reagent to room temperature before opening. The vial should not be cool to the touch. Once opened, the lyophilized material should be stored desiccated. The specific activity of the Stem Cell Dissociation Reagent is found on the product label. Prepare a 0.5 U/mL working solution by dissolving the Stem Cell Dissociation Reagent powder in the appropriate volume of DMEM: F-12 Medium (ATCC® 30-2006). Filter sterilize the solution through a 0.22 µm membrane and aliquot into working volumes according to usage. Dissolve the appropriate amount of Stem Cell Dissociation Reagent in DMEM: F-12 medium to prepare a 0.5 U/mL working solution.

Example: *To prepare 40 mL of a 0.5 U/mL working solution*:

Specific activity of Stem Cell Dissociation Reagent (on certificate of analysis): 1.46 U/mg

[(40 mL)(0.5 U/mL)]/(1.46 U/mL)= 13.7 mg

Dissolve 13.7 mg Stem Cell Dissociation Reagent in 40 mL DMEM: F-12.

Store aliquots at -20°C for up to three months. Avoid repeated freezing and thawing. Once thawed, the aliquots may be kept at 2°C to 8°C for up to two weeks.

Dissociation Protocol

This protocol is designed for the dissociation of cells in a 6-cm dish. Volumes should be adjusted according to the size and number of the tissue culture vessels to be processed.

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

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 (PBS) (ATCC® 30-2200).
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 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 with 4 mL of DMEM: F-12 Medium, taking care not to dislodge the cells during manipulation.
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 cells 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 cells 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 Inhibitor Y27632* (ATCC® ACS-3030) to the 15 mL tube.

    Note: Addition of ROCK inhibitor has been shown to increase the survival rate during subcultivation and thawing of human iPSCs. 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.[1]

14. Gently resuspend the pellet by pipetting up and down 2 to 3 times with a 1 mL tip, maintaining the small

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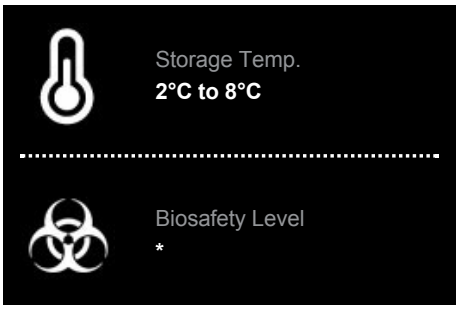

cell aggregates.

15. Plate the cells as desired on feeder or feeder-free cultures. The presence of 10 µM ROCK Inhibitor Y27632 in the stem cell culture medium is recommended.

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

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

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