ATCC

Product Sheet

Stem Cell Freezing Media (ATCC® ACS-3020™)

Please read this FIRST

Storage Temp.
2°C to 8°C

Biosafety Level
*

American Type Culture Collection
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Or contact your local distributor

Description

Product Description:
Stem Cell Freezing Media is a serum-free, xeno-free, complete medium for the cryopreservation of human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC). Stem cells preserved in this media demonstrate increased cell viability and recovery upon thawing, exhibiting consistent morphology and maintaining pluripotency. The ready-to-use solution is quick and convenient, requiring no additional supplements. Stem Cell Freezing Media is suitable for hESCs and hiPSCs cultured in both feeder-free and feeder-dependent conditions.
Volume: 1 x 20 mL

Directions for Use

Detailed protocols relating to this product are available online at
http://www.atcc.org/Portals/1/Pdf/iPSCguide.pdf.
This protocol is designed for the cryopreservation 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® 30-2006).

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)).
For optimal results, cryopreserve stem cell colonies when the cell cultures are 80% confluent.

Recommended Dissociation Protocol

1. Warm an aliquot of Stem Cell Dissociation Reagent working solution to room temperature.
2. Aspirate and discard the stem cell culture medium.
3. Rinse the cells twice with 4 mL of Dulbecco's Phosphate Buffered Saline (ATCC® 30-2200).
4. Add 2 mL of Stem Cell Dissociation Reagent working solution to the dish.
5. 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.
6. Aspirate the Stem Cell Dissociation Reagent and gently rinse the colonies with 4 mL of DMEM: F-12 Medium, taking care not to dislodge the cells during manipulation.
7. 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.
8. Transfer the cell aggregates to a 15 mL conical tube.
9. 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.
10. Centrifuge the cell aggregates at 200 x g for 5 minutes.
11. Aspirate the supernatant and discard. Gently tap the bottom of the tube to loosen the cell pellet.

Cryopreservation Protocol

1. Detach stem cell colonies from the dish as described in the recommended dissociation protocol.
2. Remove the Stem Cell Freezing Media from storage and swirl to mix. Keep cold. Decontaminate by dipping in or spraying with 70% alcohol.
3. Add 2 mL of cold Stem Cell Freezing Media to the tube containing the cell pellet. Gently resuspend the pellet by pipetting up and down 5-6 times with a 1 mL tip, maintaining the cell aggregates.
4. Immediately transfer 1 mL each of the cell suspension into two labeled cryovials.
5. Freeze the cells gradually at a rate of -1°C/min until the temperature reaches -70°C to -80°C. A freezing container (e.g., the CoolCell ®LX Alcohol-free cryopreservation container (ACS-6000)) may also be used.
6. The cells should not be left at -80°C for more than 24 to 48 hours. Once at -80°C, frozen cryovials should be transferred to the vapor phase of liquid nitrogen for long-term storage.

Handling Procedure for Frozen Cells and Initiation of Cultures

1. 30 Minutes Prior to Handling Cells - Pre-warm 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 O-ring 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 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

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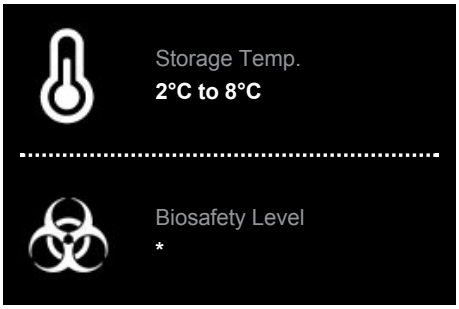

gently to mix the cells while adding media.

7. Gently pipette the cells up and down several times to mix thoroughly. Avoid breaking apart the aggregates into a single-cell 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 5 to 6 times with a 1 mL tip, maintaining the cell aggregates.
11. Plate the cells as desired under feeder-dependent or feeder-free culture conditions. The presence of 10 µM ROCK Inhibitor Y27632 in the stem cell culture medium is recommended.

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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