Stem Cell Freezing Media

ACS-3020™

Description

Stem Cell Freezing Media can be used in stem cell research, cell growth and viability, and cryopreservation of various types of human and non-human animal stem cells. This product has been qualified for use with primary-derived organoids, embryonic stem cells (hESC), induced pluripotent stem cells (hiPSC), and iPSCs-derived cells. Stem Cell Freezing Media can also be used with other continuous and cancer cell lines.

Volume: 20 mL

Storage Conditions

Storage conditions: 2°C to 8°C

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.

BSL 1

ATCC determines the biosafety level of a 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 understand the hazards associated with the material per your organization's policies and procedures as well as 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

A. Cryopreservation of human or mouse primary tissue derived organoids

We recommend freezing 100 – 200 µL of ECM containing organoids per 1 mL of freezing media. For example, if culturing organoids in 50 µL of ECM per well of a 24-well plate, 2-4 wells should be pooled and frozen in a single vial.

Procedure

1. Collect organoids and remove from ECM by mechanical dissociation.
2. Wash once in DMEM:F12 (ATCC 30-2006) and centrifuge at 500 x g for 5 minutes to pellet.
3. Aspirate supernatant.
4. Resuspend pellet in cold Stem Cell Freezing Media.
5. Immediately transfer 1 mL of the suspension to pre-labeled cryovials.
6. Place the cryovials into an ATCC CoolCell (ATCC ACS-6000) freezing container.
7. Place the freezing container at -80°C for 24 hours.
8. Remove vials from freezing container and transfer to LN_2 vapor phase for long-term storage.

B. Cryopreservation of hESC or hiPSCs

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

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: F12 Medium (ATCC 30-2006).

Stem cell culture medium

Pluripotent Stem Cell SFM XF/FF (ATCC ACS-3002) is recommended for feeder-free culture

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 once with 5 mL of DMEM:F12 (ATCC 30-2006) per 6-cm dish.
4. Add 3 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 5 mL of DMEM: F12 Medium, taking care not to dislodge the cells during manipulation.
7. Add 3 mL of stem cell culture medium to the dish, and detach the cells by pipetting up and down 3-4l 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 3 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 (eg, the CoolCell LX Alcohol-free cryopreservation container (ATCC 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 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.

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: Stem Cell Freezing Media (ATCC ACS-3020)

References

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

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