AB2.2

SCRC-1023™

Description

Organism: *Mus musculus*, mouse
Cell Type: embryonic stem cell
Tissue: Embryo; Inner cell mass
Age: embryo
Gender: Male
Morphology: Spherical colony

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

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.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

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

Growth Conditions

Temperature: 37°C

Handling Procedures

Unpacking and storage instructions:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Complete medium: Grow ES cells in Mouse ES Cell Basal Medium (ATCC SCRR-2011) that has been supplemented with the following components:

0.1 mM 2-mercaptoethanol (Invitrogen Cat. No. 21985)

1,000 U/ml mouse leukemia inhibitory factor (LIF) (EMD Millipore Cat. No. ESG1107)
15% FBS, ES Cell Qualified (ATCC SCRR-30-2020)

Complete Growth Medium for Mouse ES Cells is stable for 14 days when stored at 2°C to 8°C.
This medium is formulated for use with a 5% CO_2 in air atmosphere.
Feeder cells: Feeder cells may be grown in medium containing fewer growth factors than those required by the ES cells. Feeder cells are available from ATCC. Consult the product detail page and the product sheet provided for the feeder cells you wish to use for medium requirements. Feeder cells should be initiated 24 to 48 hours prior to inoculating with embryonic stem (ES) cells.
Handling Procedure:

Complete Medium for Feeder Cells

Feeder cells required. Feeder cells may be grown in medium containing fewer growth factors than those required by the ES cells. Feeder cells are available from ATCC. Consult the product sheet provided for the feeder cells you wish to use for medium requirements.

Feeder cells should be initiated 24-48 hours prior to inoculating with embryonic stem (ES) cells.

Feeder Cells

ATCC recommends culturing EDJ 22 on mouse embryonic fibroblasts (MEFs) that have been mitotically arrested by either irradiation or treatment with Mitomycin-C. EDJ 22 cells have been cultured on mitotically arrested MEF (CF-1) (ATCC® SCRC-1040™).

1. At least one day before plating the ES cells, prepare the desired combination of flasks with feeder cells to accommodate an initial ES cell seeding density of 30,000 cells/cm^2 to 50,000 cells/cm^2.
   Refer to the batch specific information provided on the last page of the product information sheet for the total number of viable cells recovered from this lot of ATCC® SCRC-1023™.
2. Plate mitotically arrested mouse embryonic fibroblasts (MEFs) as a feeder layer at approximately 55,000 feeder cells/cm^2 in complete medium for feeder cells.
3. Refer to the product sheet for mitotically arrested MEF for detailed handling instructions.

Feeder cells should be used within one week of plating. It is best to use feeder cells within 24-

48 hours of initiation.

Embryonic Stem (ES) Cells

1. *30 Minutes Prior to Handling Cells* – Pre-warm complete growth medium for ES cells at 37°C for at least 30 minutes before adding to cells.
2. *One Hour Prior to Thawing the ES Cells* – Perform a 100% medium change for the MEFs using complete growth medium for ES cells.
3. Thaw the vial of ES cells by gentle agitation 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 90 seconds).
4. Remove the vial from the water bath before the contents are completely thawed, and decontaminate by dipping in or spraying with 70% ethanol.
   All of the operations from this point on should be carried out under strict aseptic conditions.
5. Transfer the vial's contents plus 5 mL of complete growth medium for ES cells to a 15 mL centrifuge tube. Use an additional 1 mL of media to rinse the vial and transfer the liquid to the 15 mL tube. Add 4 mL of complete growth medium for ES cells to bring the total volume to 10 mL.
6. Spin the cells at 270 x g for 5 min. Aspirate the supernatant and resuspend the pellet in 2 mL of complete growth medium for ES cells.
7. Add the 2 mL of cell suspension to the appropriate size flask containing feeder cells and fresh compelte growth medium for ES cells (see batch specific information). ES cells should be plated at a density of 30,000 – 50,000 cells/cm^2 .
8. Incubate the culture at 37°C in a humidified 5% CO2/95% air incubator.

Routine Handling

Perform a 100% medium change every day. Passage the cells every 1 to 2 days. If the colonies are close to or touching each other the culture is overgrown. Overgrowth will result in differentiation.

Make sure that you have prepared a sufficient number of flasks pre-plated with MEF feeder layers to support frequent passage of the ES cells.

Subculturing procedure: Subculturing Procedure

Note: To insure the highest level of viability, pre-warm media and Trypsin/EDTA to

37°C before adding to cells. Volumes used in this protocol are for T75 flasks. Proportionally adjust the volumes for culture vessels of other sizes. A split ratio of 1:4 to 1:7 is recommended.

Feeder Cell Preparation for Subcultures

1. Daily maintain a sufficient number of flasks that have been pre-plated with MEFs in complete medium for feeder cells.
2. One hour before subculturing the ES cells, perform a 100% medium change for the MEFs using complete growth medium for ES cells.

Dissociation and Transfer of ES Cells

1. Aspirate the medium from the flask(s) containing ES cells.
2. Wash with PBS Ca+2/Mg+2-free (ATCC® SCRR-2201).
3. Add 3.0 mL of 0.25% (w/v) Trypsin / 0.53 mM EDTA solution (ATCC® 30-2101) and place in incubator. After about one minute the ES colonies will dissociate and all cells will detach from the flask.
4. Dislodge the cells by gently tapping the side of the flask then wash the cells off with 7-10 mL of fresh culture medium. Triturate cells several times with a 10 mL pipette in order to dissociate the cells into a single-cell suspension.
5. Spin the cells at 270 x g for 5 min. Aspirate the supernatant.
6. Resuspend in enough complete growth medium for ES cells to reseed new vessels at the desired split ratio (i.e. a split ratio of 1:4 to 1:7 is recommended). Perform a cell count to determine the total number of cells. ES cells should be plated at a density of 30,000 – 50,000 cells/ cm^2.
7. Add separate aliquots of the cell suspension to the appropriate size flask containing feeder cells and add an appropriate volume of fresh complete growth medium for ES cells to each vessel.
8. Incubate the culture at 37°C in a humidified 5% CO_2/95% air incubator. Perform a 100% medium change every day, passage cells every 1-2 days.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: AB2.2 (ATCC SCRC-1023)

References

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

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