ATCC

Product Sheet

RW.4 (ATCC® SCRC-1018™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
1

Intended Use

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

Complete Growth Medium

Grow ES cells in Mouse ES Cell Basal Medium (ATCC SCRR-2011) that has been supplemented with the following components:
1. 0.1 mM 2-mercaptoethanol (Life Technologies Cat. No. 21985-023)
2. 1,000 U/mL mouse leukemia inhibitory factor (LIF) (Millipore Cat. No. ESG1107)
3. 10% to 15% ES-Cell Qualified FBS (ATCC® SCRR-30-2020) or an ES cell qualified serum replacement
Complete Growth Medium for Mouse ES Cells is stable for 14 days when stored at 2°C to 8°C.
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.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: RW.4 (ATCC® SCRC-1018™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108, USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Mus musculus*, mouse
Strain: 129X1/SvJ
Tissue: inner cell mass
Cell Type: embryonic stem cell
Age: embryo
Gender: male
Morphology: spherical colony
Growth Properties: adherent

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials 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 vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & 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.

Handling Procedure for Frozen Cells

Complete Medium for 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 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-1018™.
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

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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 37ºC 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.

Cryopreservation Medium

Complete growth medium supplemented with an additional 10% FBS and 10% DMSO. Make immediately prior to use. Keep at 4°C.

Comments

The cells stain positive for pluripotency markers and alkaline phosphatase activity and RW.4 has been shown to be germline competent.

References

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

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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

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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.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
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Additional information on this culture is available on the ATCC web site at www.atcc.org.
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