SIM-A9

CRL-3265™

Description

SIM-A9 is a microglial cell line that was derived from cerebral cortex tissues collected and pooled from mouse pups. The tissues were dissociated by trypsinization and plated. On day 14 the microglial cells were harvested by vigorously shaking the flasks and the plating out detached microglial cells. The plates were maintained for 2 weeks at which time an unexpected, extensive proliferation of microglial cells was observed. The cells were passaged 7 times over a 4-week period and were determined to have become spontaneously immortalized. The cells were then plated out in a limited dilution procedure and colonies derived from wells observed to contain a single cell were expanded. A clone designated as “A-9” gave rise to this cell line. These spontaneously immortalized micgoglial cells exhibit phagocytic activity upon liposaccharide stimulation and TNFα cytokine secretion upon β-amyloid exposure. These cells may be used in place of primary microglial cultures, which require a lengthy and costly preparation procedure. This cell line was deposited by K Nagamoto-Combs and can be used in neuroscience and toxicology research.

Organism: *Mus musculus*, mouse
Cell Type: microglial cell
Tissue: Brain; Cerebral cortex
Age: neonate
Morphology: neuronal-like
Growth properties: Mixed: adherent and suspension
Volume: 1.0 mL

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: The base medium for this cell line is DMEM:F12 Medium Catalog No. 30-2006. To make the complete growth medium, add the following components to the base medium:

- heat-inactivated horse serum to a final concentration of 5%
- heat-inactivated fetal bovine serum to a final concentration of 10%

Handling Procedure:

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial 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 2 minutes).
2. Remove the vial from the water bath as soon as the contents are 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.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 x *g* for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator

for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Subculturing procedure:

Volumes used in this protocol are for 75 cm^2 flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Transfer all medium and floating cells from flask to a centrifuge tube.
2. Briefly rinse the cell layers with Ca^{++}/Mg^{++} free Dulbecco's phosphate-buffered saline (D-PBS). Add rinse solution to the cells in centrifuge tube in step 1.
3. Add 5.0 ml of phosphate buffered saline containing 1 mM EDTA, 1 mM EGTA and 1 mg/mL glucose solution to flask to detach cells. Observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. After the cells have detached, transfer them to the centrifuge tube in step 1.
5. Centrifuge the cells at approximately 125 x g for 5 to 10 minutes. Discard supernatant.
6. Resuspend the cell pellet in fresh growth medium.
7. Add appropriate aliquots of cell suspension to new culture vessels.
8. Place culture vessels in incubators at 37°C.

Subcultivation Ratio: 1:3 to 1:6

Medium renewal: every 2 to 3 days

Reagents for cryopreservation: Complete growth medium supplemented with 10% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: SIM-A9 (ATCC CRL-3265)

References

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

Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

Disclaimers

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. Any proposed commercial use is prohibited without a license from ATCC.

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 or complete and the customer bears the sole responsibility

of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

Copyright and Trademark Information

Revision

This information on this document was last updated on 2024-10-25

Contact Information

ATCC
10801 University Boulevard

Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor