ATCC

Product Sheet

BALB SFME Serum Free Mouse Embryo (ATCC® CRL-9392™)

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

A 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's F12 medium with 2.5 mM L-glutamine, 1.2 g/L sodium bicarbonate, 15mM HEPES and 0.5mM sodium puruvate supplemented with: 0.010 mg/ml bovine insulin 0.010 mg/ml human transferrin 1% chemically defined lipids (LifeTechnologies cat.# 11905-031) 50 ng/ml mouse epidermal growth factor 10 nM sodium selenite

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: BALB SFME Serum Free Mouse Embryo (ATCC® CRL-9392™)

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: BALB/c
Tissue: embryo
Disease: normal
Cell Type: Astrocyte
Age: embryo; 16 days
Morphology: fibroblast
Growth Properties: loosely adherent
Cytogenetic Analysis: modal number = 40

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

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 growth medium and spin at approximately 125 x g for 5 to 7 minutes. Discard supernatant.
4. Resuspend the cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. 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).
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.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information), grown, and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached,** aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL

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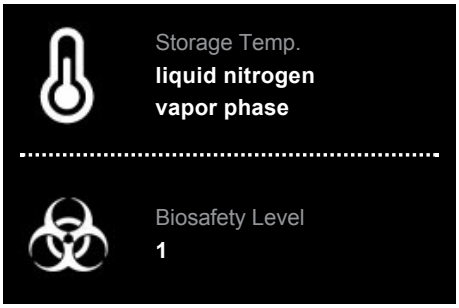

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of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.
Note: Coat culture vessels with 0.01 mg/mL fibronectin solution **before** adding cells. Add culture medium plus an equal volume of 0.01 mg/mL fibronectin solution sufficient to cover the flask surface; immediately mix by rocking (Do not premix medium with fibronectin). Incubate at 37°C for at least 30 minutes. Suction to remove excess liquid prior to adding cells. Pre-coated culture vessels can be stored with medium for several days in the incubator.

1. Attached cells may be removed with 0.05% trypsin - 0.53mM EDTA. The cells are very sensitive to trypsin; therefore the trypsinization process should take only a few seconds.
2. Immediately inhibit the trypsin with equal volume of cold soybean trypsin inhibitor (0.1%) and dilute the cell suspension 10 fold with cold medium.
3. Pellet cells, resuspend in fresh medium and dispense into new **pre-coated** flasks. Non-attached cells can be spun down and replated in pre-coated flasks.
4. Initially plate cells with half volume of medium in 5% CO_2 in air atmosphere, wait 2-3 hours, and then add additional volume of medium.
   Note: The cells are very density and temperature sensitive. Confluencies above 80% will cause cells to form clumps and detach.

DO NOT LEAVE FLASKS AT ROOM TEMPERATURE OR CELLS WILL DETACH.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:10 is recommended
Medium Renewal: Three to four times weekly with pre-warmed medium

Cryopreservation Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

BALB/c SFME cells infrequently form colonies in soft agar and are not tumorigenic if injected into nude mice. Either serum or TGF-beta induce astrocyte differentiation accompanied by GFAP (glial fibrillary acidic protein) expression.
The presence of serum causes cell growth arrest. This process is reversible upon removal of the serum. When cultured in serum-free medium, BALB SFME cells reportedly can be propagated for extended periods without undergoing crisis or gross chromosomal aberration.

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

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. 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 product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

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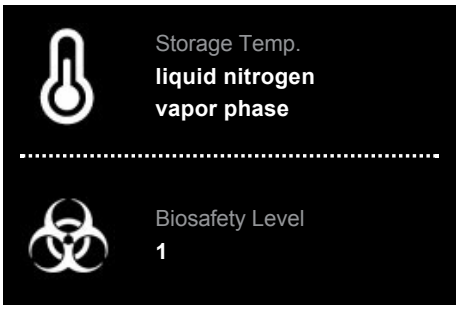

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