OP9

CRL-2749™

Description

OP9 is a macrophage-derived embryonic stem cell line that was isolated from the stroma of a mouse embryo. This cell line was deposited by T Nakano and can be used in stem cell research.

Organism: *Mus musculus*, mouse
Cell Type: embryonic stem cell, macrophage-derived
Tissue: Bone; Marrow; Stroma
Age: embryo
Morphology: fibroblast-like
Growth properties: Adherent

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
Atmosphere: 95% Air, 5% CO_2

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 Alpha Minimum Essential Medium without ribonucleosides and deoxyribonucleosides and with 2.2 g/L sodium bicarbonate. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 20%

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. It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.
4. Transfer the cells to an appropriate size vessel. 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 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.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in Culture Of Aminal Cells: A Manual of Basic Techniques by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.

Subculturing procedure: Volumes are given for a 75 cm^2 flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

Note: Cell density is important. If the subculture ratio is too low, the culture will not reach confluence. However, do not overgrow. Very large cells tend to appear after overgrowth and these cells are a warning sign that the OP9 cells will not support the maintenance of hematopoietic cells. Subculture just before confluence.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and 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. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 125 X g for 5 to 10 minutes. Discard supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 4 X 10^3 and 1 X 10^4 cells/cm^2.
Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:5 is recommended
Medium Renewal: Every 2 to 3 days
Reagents for cryopreservation: Complete growth medium supplemented with 5% (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: OP9 (ATCC CRL-2749)

References

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

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Revision

This information on this document was last updated on 2024-06-15

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