OP9

305174

GENERAL INFORMATION

Description

The OP9 cell line, a stromal cell line derived from the calvariae of op/op mice, has a mutation that leads to a lack of macrophage colony-stimulating factor (M-CSF), which is a critical cytokine involved in the differentiation, survival, and function of various cell types, including macrophages and osteoclasts.
OP9 cells have been extensively used in the field of hematopoiesis research as feeder layers in co-culture systems to support the differentiation and expansion of both hematopoietic stem cells (HSCs) and embryonic stem cells (ESCs). These co-culture systems have facilitated the study of hematopoietic differentiation pathways, enabling MSCs to differentiate into adult erythroid cells, erythroblasts, and red blood cells and osteocytes, chondrocytes, myocytes, tenocytes, and adipocytes. The supportive role of OP9 cells in these systems is attributed to their ability to produce a conducive microenvironment rich in cytokines and growth factors essential for stem cell proliferation and lineage-specific differentiation.
Furthermore, the OP9 cell line is instrumental in studying the leukocyte reaction and the development of immune cells such as natural killer (NK) cells, demonstrating the OP9 mouse line's utility in immunological research. The secretory factors produced by OP9 cells, including growth factors like bFGF, IGF-1, IL-3, PDGF-BB, TGF-β1, and TGF-β3, play a critical role in cell migration and differentiation processes.
OP9 cells exhibit a fibroblast-like appearance, characterized by a spindle-shaped, flat morphology. This morphological trait is typical of mesenchymal stromal cells, which are known for their supportive functions in the bone marrow microenvironment.
Despite their vast potential, OP9 cells have limitations due to their non-immortalized nature, which confines their use to short-term and small-scale projects, underscoring the need for careful planning and consideration in experimental designs.

Tissue	Bone marrow, stroma

Synonyms

- OP-9

CLASSIFICATION

Organism	Mouse
Growth properties	Adherent
Biosafety level	1

CHARACTERISTICS

Morphology	Fibroblast-like

IDENTIFIERS

Citation

OP9 (Cytion catalog number 305174)

NCBI TaxID	10090
Cellosaurus	CVCL_4398

HANDLING AND CULTURE

Culture medium

Alpha MEM, w: 2.0 mM stable Glutamine, w/o: Ribonucleosides, w/o: Deoxyribonucleosides, w: 1.0 mM Sodium pyruvate, w: 2.2g/L NaHCO3

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Incubation atmosphere	37°C, 5% CO_2, humidified atmosphere.

Supplements

Supplement the medium with 20% FBS

Dissociation reagent	Accutase

Subculturing

Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.

Fluid renewal	2 to 3 times per week

Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

QUALITY CONTROL AND CRYOPRESERVATION

Sterility

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.
To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.

Freeze medium	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

Storage conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Shipping conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

DONOR

Breed / subspecies	(C57BL/6 x C3H) F2-op/op
Donor age	Embryo

LEGAL AND SAFETY

Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

Material Transfer Agreement required	Yes

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