Data Sheet

Mouse OP9-DL4 Delta-like 4 Notch Ligand Cell Line

SCC494

Pack Size: ≥ 1x10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for human or animal consumption.

Background

OP9-DL4 is a stable bone marrow derived stromal cell line expressing the Notch ligand- Delta like 4 (DL4) ectopically.

Notch signaling controls multiple cell fate decisions. Four Notch receptors (Notch 1--- Notch 4) have been identified in mammals. These receptors interact with Jagged or Delta-like (DL) family members of Notch ligands. This is followed by cleavage of the intracellular domain of Notch and its subsequent translocation into the nucleus, where it binds with transcription factors and activates transcription of various downstream target genes.[1] Notch signaling is essential in early T- cell lineage commitments.[2] Bone marrow progenitor cells expressing constitutively active Notch develop into CD4 and CD8 double positive T cells rather than B-cells. They also play an important role in development of $CD4^+$ and $CD8^+$ single positive cells from double positive precursor thymocytes, in addition to development of T-cells with $\alpha\beta^+$ TCRs.

Of the 4 Notch receptors, Notch1 (N1) receptor signaling has been shown to be sufficient for T cell development. The delta-like ligands are physiologically relevant N1 ligands. DL1 interacts with both N1 and Notch 2 (N2) to induce T- cell lineage commitment. However, DL4 interacts specifically with N1 only, and supports T cell commitment and maturation both *in vitro* and *in vivo*. Moreover, results from previous binding studies show that binding between DL1 and N1 is weak compared with the stronger binding between DL4 and N1.[3] Of the DL1 and DL4 delta-like ligands, DL4 exhibits greater capacity to activate the Notch pathway in hematopoietic progenitor cells.[4]

OP9 bone marrow stromal cells support the differentiation of hematopoietic progenitor cells (HPCs) into multiple lineages including B-cells, but not to T cells. This is mainly because they do not express Delta-like ligands. Therefore, OP9 cells were transduced with a pMigR retroviral vector engineered to express the Delta-like-4 gene along with green fluorescent protein (GFP). The OP9-DL4 cells were then sorted based on GFP expression.[1] These OP9-DL4 cells support the differentiation of embryonic or hematopoietic stem cells from fetal liver or bone marrow to T lymphocytes.

Source

OP9-DL4 cells were genetically modified from OP9 stromal cells derived from mouse bone marrow.

Short Tandem Repeat

M18-3: 16	M1-2: 17	M8-1: 17	M11-2: 16	MX-1: 28
M4-2: 19.3, 20.3	M7-1: 26.2	M2-1: 15, 16	M17-2: 15	M13-1: 17
M6-7: 17	M1-1: 16, 17	M15-3: 22.3, 26.3	M12-1: 17	
M19-2: 13	M3-2: 13, 14	M6-4: 18	M5-5: 15	

Quality Control Testing

- OP9-DL4 murine bone marrow stromal cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and nonhuman primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases against a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

OP9-DL4 cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

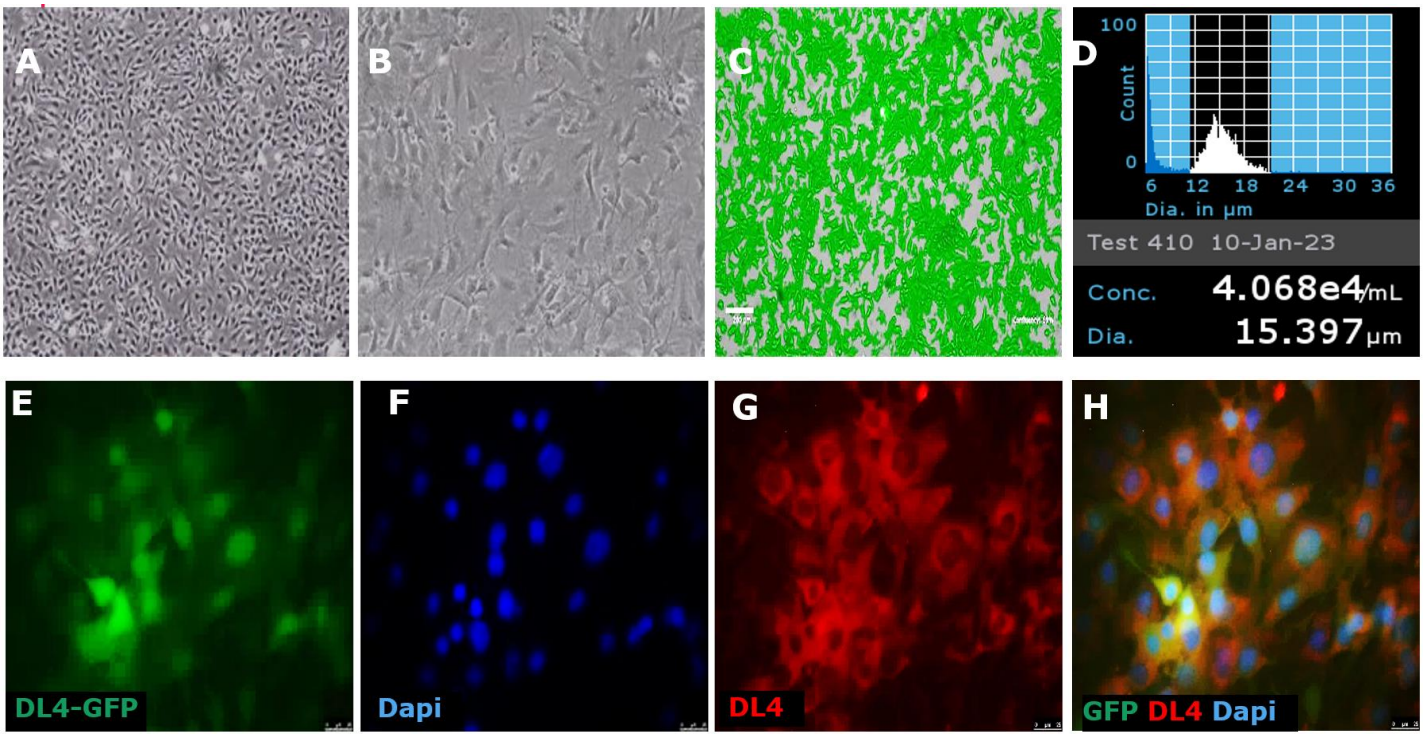

Figure 1. **A**. **B**. (4X and 10X magnification) Bright-field images of OP9-DL4 cells two days after thaw in a T75 flask. **C**. (MDCI10000) Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager. **D**. (PHCC360KIT) Cell counting was performed using the Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensors. **E**. OP9-DL4 cells are GFP positive and **G**. (HPA023392) express DL4 protein. **H**. Merged images.

NOTE: Product catalog numbers indicated in ( ) can be purchased at SigmaAldrich.com unless otherwise stated.

Protocols

OP9-DL4 cells proliferate slowly with an approximate doubling time of 5-6 days.

Thawing the Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on standard tissue cultureware surfaces without any additional coating.

   OP9-DL4 cells are thawed and expanded in OP9-DL4 Expansion Medium comprising of MEM-alpha (M4526) containing 5% FBS (ES-009-B), 2 mM L-Glutamine (G7513) and Penicillin/Streptomycin (P4333) (optional).
2. Remove the vial of frozen OP9-DL4 cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1- or 2-mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of OP9-DL4 cells Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of OP9-DL4 Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. OP9-DL4 cells can be passaged at ~80-85% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80-85% confluent layer of OP9-DL4 cells.
3. Rinse the flask with 10 mL 1X sterile PBS (TMS-012-A). Aspirate after the rinse.
4. Apply 5-7 mL of pre-warmed Accutase® (A6964) and incubate in a 37 °C incubator for 5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of OP9-DL4 Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of OP9-DL4 cell medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.

    IMPORTANT: Do not vortex the cells.
11. Count the number of cells using a hemocytometer or a Scepter™ 3.0 handheld automated cell counter.
12. Plate the cells to the desired density. Typical split ratio is 1:5.

Cryopreservation of the Cells

OP9-DL4 cells may be frozen in OP9-DL4 Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. Immunity 2002, 17(6): 749-756.
2. Curr Opin Immunol. 2007, 19(2): 163-168.
3. J Exp Med. 2007, 204(2):331-343.
4. J Immunol. 2010, 185(2):867-876.

Academic Use Agreement

Subject to local law

THIS PRODUCT MAY ONLY BE USED BY INDIVIDUALS EMPLOYED BY AN ACADEMIC INSTITUTION AND IS INTENDED SOLELY TO BE USED FOR ACADEMIC RESEARCH, WHICH IS FURTHER DEFINED BELOW. BY OPENING THIS PRODUCT, YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR YOUR EMPLOYER INSTITUTION, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THE TERMS OF THIS ACADEMIC USE AGREEMENT. IF YOU DO NOT AGREE TO COMPLY WITH THESE TERMS, YOU MAY NOT OPEN OR USE THE PRODUCT AND YOU MUST CALL ("SELLER") CUSTOMER SERVICE (1-800-645-5476) TO ARRANGE TO RETURN THE PRODUCT FOR A REFUND.

"Product" means Mouse OP9-DL4 Delta-like 4 Notch Ligand Cell Line (SCC494).

"Academic Research" means any internal in vitro research use by individuals employed by an academic institution. Academic Research specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the development, testing, or manufacture of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product in clinical or therapeutic applications including producing materials for clinical trials
- Administering the Product to humans
- Using the Product in collaboration with a commercial or non-academic entity

"Commercial Product" means any product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

Access to the Product is limited solely to those officers, employees, and students of PURCHASER's academic institution who need access to the Product to perform Academic Research. PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access.

These use restrictions will remain in effect for as long as PURCHASER possesses the Product.

COMMERCIAL OR NON-ACADEMIC ENTITIES INTERESTED IN PURCHASING OR USING THE PRODUCT MUST CONTACT licensing@emdmillipore.com AND AGREE TO SEPARATE TERMS OF USE PRIOR TO USE OR PURCHASE.

Genetically Modified Organisms (GMO)

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient des organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Document Template 20306518 Ver 6.0
00149643 Ver 1.0, Rev 21NOV2023, RC, AB