Data Sheet

Mouse OP9-DL4-7FS Notch Ligand Cell Line

SCC490

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. They 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 the transcription factors and activates transcription of various downstream target genes.[1]

Notch signaling is essential in early T-cell lineage commitments. Bone marrow progenitor cells expressing constitutively active Notch have been shown to develop into CD4 and CD8 double positive T cells rather than B-cells. They also play a key role in development of CD4$^+$ and CD8$^+$ single positive cells from CD4 and CD8 double positive precursor thymocytes. Also, this signaling has been shown to promote development of T-cells with αβ+ TCRs. Thus, Notch signaling is critical for the earliest stages of T-cell commitment.[2]

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

The OP9 bone marrow stromal cells that support the differentiation of hematopoietic progenitor cells (HPCs) into multiple lineages including B-cells, failed to support differentiation of HPCs to T-cells. This is mainly because they do not express Delta-like ligands. Therefore, OP9 cells were transduced with pMiGR retroviral vector engineered to express the Delta-like-4 gene. These OP9-DL4 cells support the differentiation of embryonic or hematopoietic stem cells from fetal liver or bone marrow into T lymphocytes.

Human hematopoietic stem cells (HSCs) require additional cytokines for survival and propagation. Three important components are added separately to HSC/OP9-DL cocultures. These components are Interleukin-7 (IL-7), stem cell factor (SCF), and FMS-like tyrosine kinase 3 ligand (FLT3L). To circumvent the costly addition of these cytokines, an improved OP9-DL4 cell line was designed and designated OP9-DL4-7FS.5 This improved OP9 cell line was transduced to express human IL-7, FLT3L, and SCF (7FS), making it a highly cost-efficient cell line and improved version of the original OP9-DL4 cell line. In addition, the cerulean fluorescent protein (CeFP) gene was substituted for GFP using restriction enzymes to distinguish the new "7FS" cell line from the original OP9-DL4 cell line which had been sorted for GFP expression.

Source

Parental 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, 29
M4-2:	20.3	M7-1:	26, 26.2	M2-1:	15, 16	M17-2:	12, 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, 19	M5-5:	15		

Spectral Properties

- Expresses cerulean fluorescent protein (CeFP)
- Excitation: 433 nm
- Emission: 475 nm

Quality Control Testing

- The OP9-DL4-7FS 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

The OP9-DL4-7FS 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

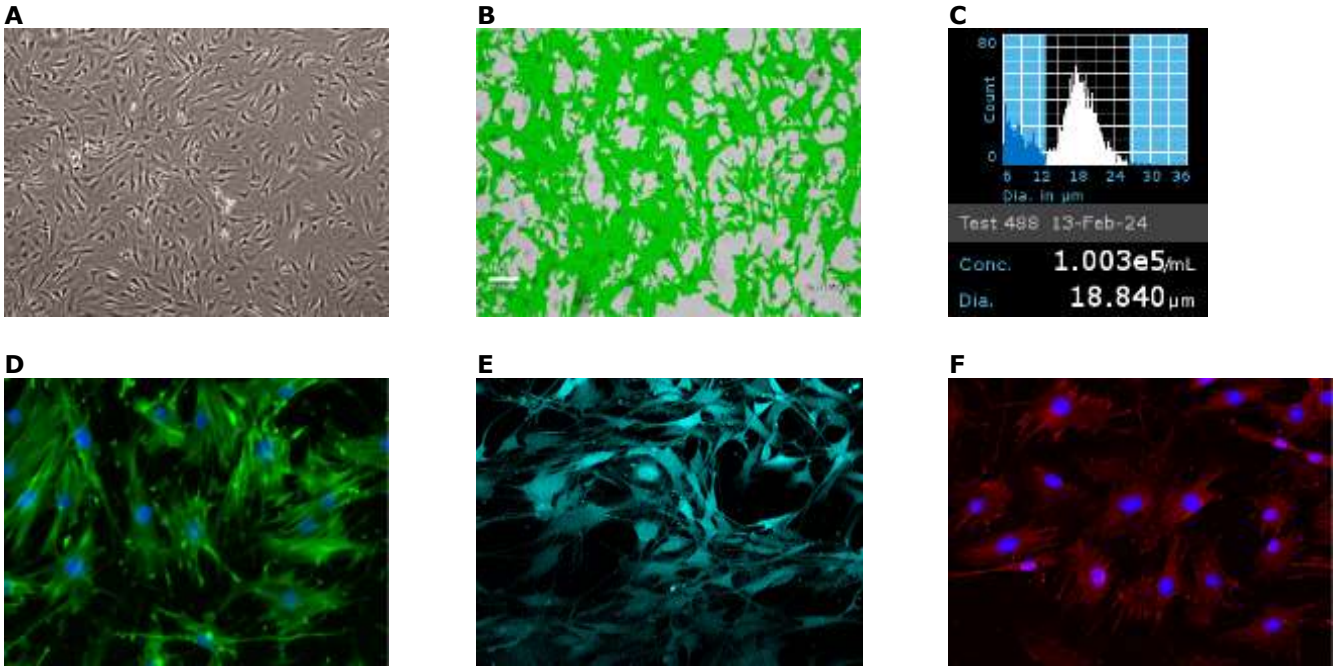

Figure 1. (**A**) Bright-field image of OP9-DL4-7FS cells one day after thaw in a T175 flask (4X magnification). (**B**) Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). (**C**) Cell counting was performed using the Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensors (PHCC360KIT). (**D**) The OP9-DL4-7FS cells stained with Phalloidin-Atto-647N (65906). (**E**) OP9-DL4-7FS cells express cerulean fluorescent protein (CeFP shown in cyan). (**F**) The OP9-DL4-7FS cells express DL4 protein (HPA023392).
Note: Product catalog numbers indicated in () can be purchased at SigmaAldrich.com unless otherwise stated

Protocols

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.
2. The OP9-DL4-7FS cells are thawed and expanded in OP9-DL4 Expansion Medium comprising of MEM-Eagle (M4526) containing 5% FBS (ES-009-B), 2 mM L-Glutamine (G7513) and Penicillin/Streptomycin (P4333) (optional).
3. Remove the vial of frozen OP9-DL4-7FS 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.
4. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
5. In a laminar flow hood, use a 1-2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
6. Using a 10 mL pipette, slowly add dropwise 9 mL of OP9-DL4 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.

7. 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.
8. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
9. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
10. Resuspend the cells in 35 mL of OP9-DL4 Expansion Medium.
11. Transfer the cell mixture to a T175 tissue culture flask.
12. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. The OP9-DL4-7FS cells can be passaged at ~80-85% confluency.
2. Carefully remove the medium from the tissue culture flask containing the 80-85% confluent layer of OP9-DL4-7FS 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 1:6.

Cryopreservation of the Cells

The OP9-DL4-7FS cells may be frozen in OP9-DL4 Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Schmitt TM and Zúñiga-Pflücker JC. 2002. Induction of T cell development from hematopoietic progenitor cells by delta-like-1 in vitro. Immunity. 17(6): 749–756.
2. de Pooter R and Zúñiga-Pflücker JC. 2007. T-cell potential and development in vitro: the OP9-DL1 approach. Curr Opin Immunol. 19(2):163–168.
3. Besseyrias V, Fiorini E, Strobl LJ, Zimber-Strobl U, Dumortier A, Koch U, Arcangeli M-L, Ezine S, Macdonald HR, Radtke F. 2007. Hierarchy of Notch–Delta interactions promoting T cell lineage commitment and maturation. J Exp Med. 204(2):331–343.
4. Mohtashami M, Shah DK, Nakase H, Kianizad K, Petrie HT, Zúñiga-Pflücker JC. 2010. Direct comparison of Dll1- and Dll4-mediated Notch activation levels shows differential lymphomyeloid lineage commitment outcomes. J Immunol. 185(2):867–876.
5. Mohtashami M, Brauer PM, Zúñiga-Pflücker JC. 2023. Induction of human T cell development in vitro with OP9-DL4-7FS cells expressing human cytokines. Methods Mol Biol. 2580:249–260.

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