Data Sheet

OP9-DL1 hDL-1-Expressing mOP9 Cell Line

Murine bone marrow derived stromal cell line

SCC136

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-DL1 is a stable bone marrow-derived stromal cell line expressing the Notch ligand Delta-like I (DL1) ectopically.

Notch signaling controls multiple cell fate decisions. It is initiated via engagement of the Notch receptor with its ligands (Jagged or Delta-like family members). This is followed by cleavage of the intracellular domain of Notch and its subsequent translocation into the nucleus, where it binds with the transcription factor CBF-1/RBPJ and activates transcription of various downstream target genes.[1] Upregulation of Notch ligands is also a factor in chronic inflammatory diseases including pancreatitis and diabetic nephropathy.[2] Several studies have implicated the role of Notch signaling in early T cell lineage commitments as it promotes development of T cells with αβ+ T Cell Receptors (TCRs) at the expense of γδ+ TCRs.[3] Bone marrow progenitor cells expressing constitutively active Notch have been shown to develop into CD4 and CD8 double positive T cells. This signaling pathway also plays an important role in development of CD4$^+$ or CD8$^+$ single positive cells from CD4 and CD8 double positive precursor thymocytes.

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 1 or Delta-like 4 notch ligands endogenously.

OP9 cells were therefore infected with a MiGR1 retroviral vector engineered to express the human Delta-like-1 gene and green fluorescent protein (GFP). The OP9-DL1 cells were then sorted for GFP expression.[1] OP9-DL1 cells support the differentiation of embryonic or hematopoietic stem cells from fetal liver or bone marrow into T lymphocytes. Holmes *et al* have provided a detailed protocol to differentiate embryonic or hematopoietic stem cells into T lymphocytes using the OP9-DL1 system.[4] The OP9-DL1 cell line is an important tool for the exploration of the role of Notch signaling in cellular differentiation and in the genesis of inflammatory disease states.

Source

GMO. OP9-DL1 cells were genetically modified from 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: 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-DL1 murine bone marrow stromal cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and non-human 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-DL1 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

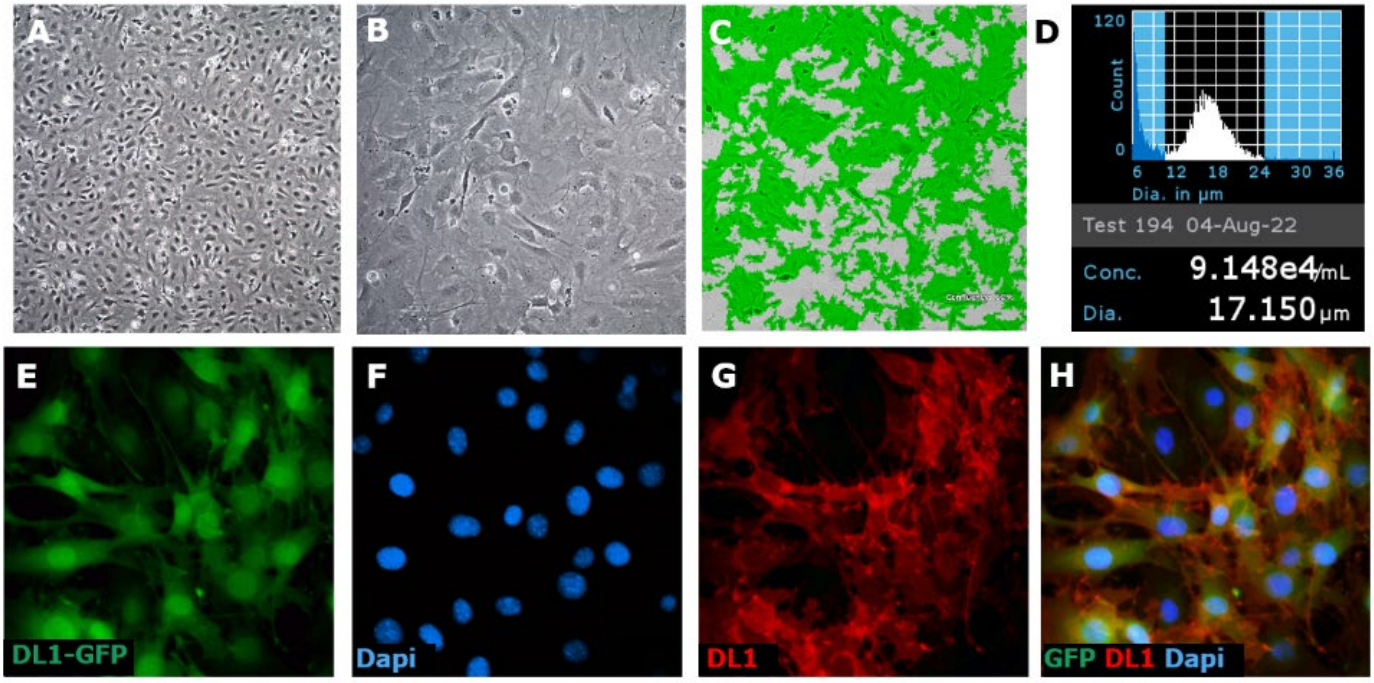

Figure 1. Brightfield images of OP9-DL1 cells one day after thaw in a T75 flask (4X and 10X magnification, **A**, **B**). Cell confluency was assessed throughout the culture using the MilliCell® Digital Cell Imager (**C**, Cat. No. MDCI10000). Cell counting was performed using Scepter™ 3.0 handheld automated cell counter using 60 µm sensors (**D**, Cat. No. PHCC360KIT). OP9-DL1 cells are GFP positive (**E**) and express DL1 protein (**G**, Cat. No. MABN2284). Merged images (**H**).

Protocols

OP9-DL1 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-DL1 cells are thawed and expanded in OP9-DL1 Expansion Medium comprising of MEM-alpha (Cat. No. M4526) containing 5% FBS (Cat. No. ES-009-B), 2 mM L-Glutamine (Cat. No. G7513) and Penicillin/Streptomycin (Cat. No. P4333) (optional).
2. Remove the vial of frozen OP9-DL1 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-DL1 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-DL1 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-DL1 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-DL1 cells.
3. Rinse the flask with 10 mL 1X sterile PBS (Cat. No. TMS-012-A). Aspirate after the rinse.
4. Apply 5-7 mL of pre-warmed Accutase® reagent (Cat. No. 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-DL1 Expansion Medium to the flask.
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-DL1 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-DL1 cells may be frozen in OP9-DL1 Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. *Immunity* 2002, 17(6): 749-756.
2. *J Orthop Sci*. 2005, 10(6): 589-594.
3. *Curr Opin Immunol*. 2007, 19(2): 163-168.
4. *Cold Spring Harb Protoc*. 2009, 2009(2): pdb.prot5156.

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