MS5-hDLL1 Stromal Cell Line

Immortalized Cell Line

Cat. # SCC167

Pack size: ≥1x10^6 viable cells/vial

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

Store in liquid nitrogen

Data Sheet

Background:

In vivo, T-cells develop in the thymus from bone-marrow derived hematopoietic stem and progenitor cells (HSPCs). *In vitro*, various model systems have been employed to mimic thymic function supportive of T-cell development. The classic OP9-DL1 culture system utilizes a coculture of HSPC with a murine bone marrow-derived stromal cell line (OP9) that ectopically expresses the Notch ligand, Delta-like 1 (Dll1). The OP9-DL1 culture system utilizes fetal calf serum and thus contributes to variable efficiencies in T-cell differentiation and moreover suffers from low maturation rates. Similarly, three-dimensional (3D) organoid systems utilizing primary thymic tissues from murine[1,2,3] or human[4] sources suffer from experimental variability and difficulty in access of primary tissues.

Recently, a serum-free artificial thymic organoid (ATO) system[5] was developed that supports highly efficient and reproducible *in vitro* differentiation and positive selection of human T-cells from multiple sources of HSPCs, including cord blood, bone marrow and peripheral blood CD34+ HSPCs. A key component of the ATO system is the MS5-hDLL1 cell line which obviates the need for primary thymic tissues. To form organoids, MS5-hDLL1 stromal cells are aggregated with HSPCs by centrifugation and cultured on a cell culture insert at the air-fluid interface[5]. Detailed protocols for setting up the artificial thymic organoid (ATO) system are described in reference 5.

To generate the MS5-hDLL1, a murine stromal cell line (MS5)[6] was transduced with a third-generation lentiviral vector encoding human DLL1 and eGFP[5]. The highest 5% GFP-expressing cells were FACS-sorted and expanded.

Storage and Handling

MS5-hDLL1 cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are negative for mycoplasma contamination.
- Cells are tested negative for infectious diseases by a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of mouse origin and negative for inter-species contamination from rat, chinese hamster, Golden Syrian hamster, human and non-human primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services

Representative Data

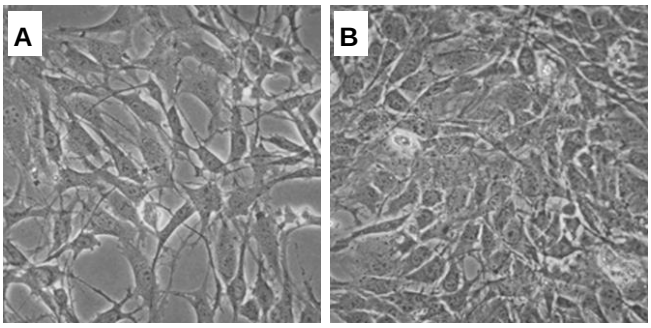

Figure 1. MS5-hDLL1 cells one (A) and two (B) days after thawing in a T75 flask.

References

1. Anderson G, Jenkinson EJ, Moore NC, Owen JJ (1993) MHC class II-positive epithelium and mesenchyme cells are both required for T-cell development in the thymus. Nature 362(6415): 70-73.
2. Plum J, De Smedt M, Defresne MP, Leclercq G, Vandekerckhove B (1994) Human CD34+ fetal liver stem cells differentiate to T cells in a mouse thymic microenvironment. Blood 84(5): 1587-1593.
3. Poznansky MC, et al. (2000) Efficient generation of human T cells from a tissue-engineered thymic organoid. Nat Biotech 18(7): 729-734.
4. Chung B, et al. (2014) Engineering the human thymic microenvironment to support thymopoiesis in vivo. Stem Cells 32(9): 2386-2396.
5. Seet CS, et al. (2017) Generation of mature T cells from human hematopoietic stem and progenitor cells in artificial thymic organoids. Nat Methods 14(5): 521-530.
6. Itoh K, et al. (1989) Reproducible establishment of hematopoietic supportive stromal cell lines from murine bone marrow. Exp Hematol 17(2): 145-153.

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Protocols

Thawing Cells

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

   Cells are thawed and expanded in High Glucose DMEM (Sigma Cat. No. D6429), 10% FBS (Cat. No. ES-009-B), and 1X Penicillin-Streptomycin Solution (Cat. No. TMS-AB2-C) (optional).
2. Remove the vial of frozen MS5-hDLL1 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 MS5-DLL1 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 45 – 50 mL of MS5-DLL1 Expansion Medium.
10. Transfer the cell mixture to a **T175** tissue culture flask.

    Note: *MS5-hDLL1 cells proliferate rapidly.*
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.

Subculturing Cells

1. Carefully remove the medium from the T175 tissue culture flask containing the confluent layer of MS5-hDLL1 cells.
2. Rinse the T175 flask twice with 15-20 mL 1X PBS. Aspirate after each rinse.
3. Apply 10-15 mL of Accutase or trypsin-EDTA solution and incubate in a 37°C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 12 mL of MS5-DLL1 Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 50 mL conical tube.
7. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2-5 mL of MS5-DLL1 Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer.
11. Plate the cells to the desired density (typical split ratio is 1:6- 1:9). Cells proliferate extremely rapidly.

Cryopreservation of Cells

MS5-hDLL1 mouse bone marrow stromal cell line transduced with human DLL1 may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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