DC2.4 Mouse Dendritic Cell Line

Immortalized Cell Line

Cat. # SCC142

Pack size: ≥1X10^6 viable cells/vial

Store in liquid nitrogen

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Data Sheet

Background

Dendritic cells (DC) are the antigen presenting cells of the immune system and are found in most tissues, particularly those that are in contact with the external environment (e.g., skin and the inner linings of the nose, lungs, stomach and intestine). First described in 1973[1], one of the primary functions of DCs is to phagocytose foreign pathogens and present the processed antigens to naïve T cells to regulate adaptive immune responses. DCs also express Toll-like receptors and help regulate the innate immune responses. Despite their distribution in most tissues, DC are present at low numbers *in vivo* and are difficult to maintain *in vitro*. These difficulties have limited the studies of dendritic cells.

DC2.4 are immortalized murine dendritic cells created by transducing bone marrow isolates of C57BL/6 mice with retrovirus vectors expressing murine granulocyte-macrophage CSF (GM-CSF) and the *myc* and *raf* oncogenes[2]. DC2.4 exhibits characteristic features of dendritic cells including cell morphology and the expression of dendritic cell-specific markers and the ability to phagocytose and present exogenous antigens on both MHC class I and class II molecules[2].

Storage and Handling

DC2.4 Mouse Dendritic Cell Line 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 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.
- Cells are negative for mycoplasma contamination

Representative Data

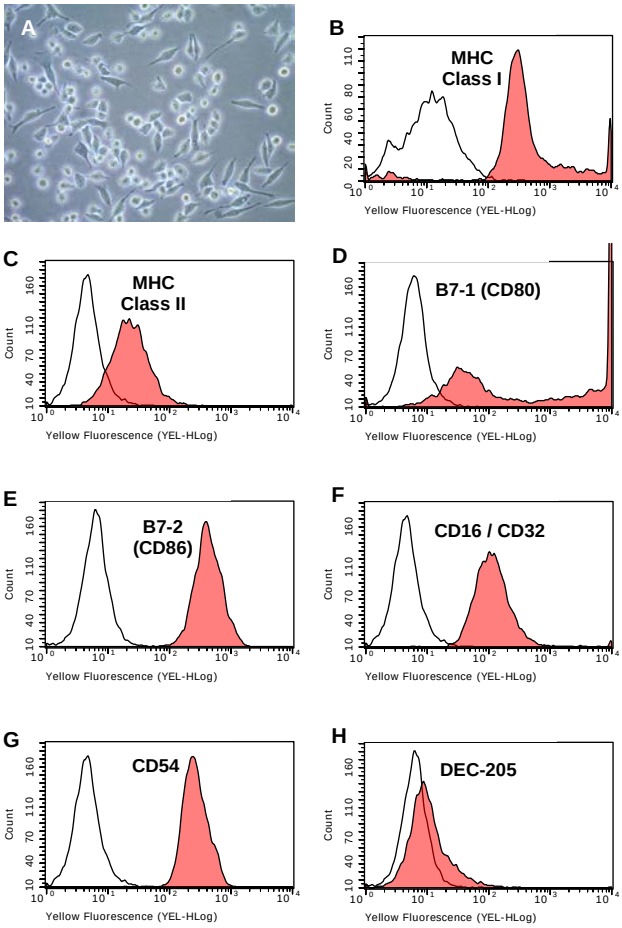

Figure 1. Day 1 after thaw (**A**). Flow analysis of cell surface molecules. DC2.4 cells express high levels of MHC class I (**B**) and class II molecules (**C**), costimulatory molecules B7-1 (**D**) and B7-2 (**E**), as well as CD32 (FcγRII) (**F**) and CD54 (**G**). The dendritic marker DEC-205 (**H**) was less expressed.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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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 RPMI-1640 (Sigma Cat. No. R0883), 10% FBS (Cat. No. ES-009-B), 1X L-Glutamine (Cat. No. TMS-002-C), 1X non-essential amino acids (Cat. No. TMS-001-C), 1X HEPES Buffer Solution (Cat. No. TMS-003-C) and 0.0054X β-Mercaptoethanol (Cat. No. ES-007-E).
2. Remove the vial of frozen DC2.4 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 DC2.4 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 xg 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 10-15 mL of DC2.4 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.
12. The next day, exchange the medium with 10-15 mL of fresh DC2.4 Expansion Medium. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 80-85% confluent, they can be dissociated with Accutase (Cat. No. SCR005) or trypsin-EDTA (Cat. No. SM-2003-C) and further passaged or, alternatively, frozen for later use.

Subculturing Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of DC2.4 cells.
2. Rinse the T75 flask twice with 10 mL 1X PBS. Aspirate after each rinse.
3. Apply 3-5 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 8 mL of DC2.4 Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 xg 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 mL of DC2.4 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 – 1:10).

Cryopreservation of Cells

DC2.4 Immortalized Murine Dendritic Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. Steinman RM, Cohn ZA (1973) Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* 137(5): 1142-1162.
2. Shen Z, Reznikoff G, Dranoff G, Rock KL (1997) Cloned dendritic cells can present exogenous antigens on both MHC class I and class II molecules. *J Immunol* 158(6): 2723-2730.

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