IMG Mouse Microglial Cell Line

Immortalized Cell Line

Cat. # SCC134

Pack size: ≥1X10^6 viable cells/vial

Store in liquid nitrogen

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THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

Certificate of Analysis

Background

Microglia are the primary immune cells of the central nervous system (CNS), and account for 10-15% of all cells found within the brain. Microglial cells play an important role in maintaining the health of the CNS by the removal of pathogens, infectious agents and damaged cells through phagocytosis. Microglial cells have also been extensively studied for their harmful roles in a variety of neurodegenerative disease including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.

Immortalized **M**icro**G**lial Cell Line or IMG is an immortalized microglial cell line derived from adult murine brain infected with the v-raf/v-myc retrovirus. IMG cells express markers that are specific to adult primary microglial cells (F4/80 and CD11b) as assessed by flow cytometry and are responsive to pro- and anti-inflammatory signals[1]. They have been shown to phagocytose foreign particles and misfolded proteins such as the amyloid-beta peptide[1]. IMG cells are rapidly dividing cells that are typically small and round with few or no processes when cultured in medium containing serum.

Storage and Handling

IMG Mouse Microglial 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.

Data

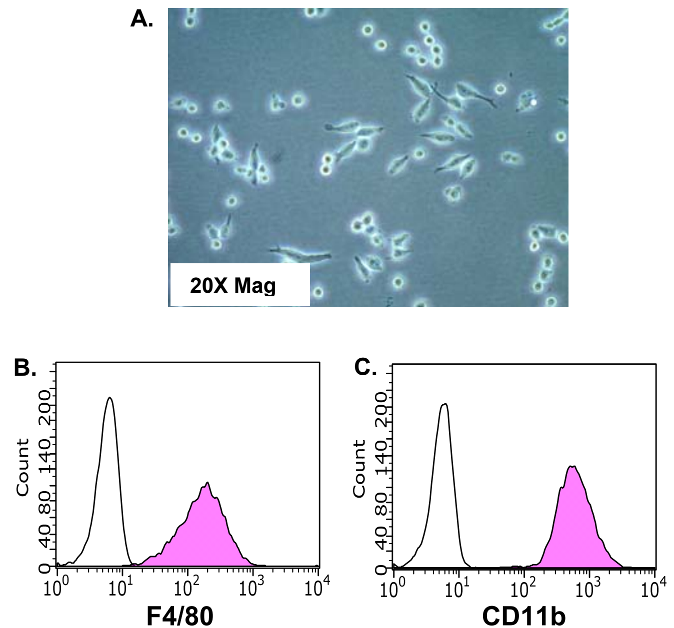

Figure 1. IMG cells exhibit similar morphology to primary microglia (**A**) and express microglial markers F4/80 (**B**) and CD11b (**C**). IMG cells were stained with anti-F4/80 (mouse), APC clone BM8.1 (Cat No. MABF1523; filled trace) (**B**). Rat IgG2a negative control, clone 2A3, APC conjugate (Cat. No. MABF1774; open trace) was used as the isotype control. IMG cells were stained with anti-CD11b (human/mouse), APC clone M1/70 (Cat. No. MABF520; filled trace) (**C**). Rat IgG2b negative control, clone LTF-2, APC conjugate (Cat. No. MABF1772; open trace) was used as the isotype control.

References

1. McCarthy RC, Lu DY, Alkhateeb A, Gardeck AM, Lee CH and Wessling-Resnick M (2016). Characterization of a novel adult murine immortalized microglial cell line and its activation by amyloid-beta. *J. Neuroinflammation* 13: 21

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

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.

   IMG Expansion Medium: Cells are thawed and expanded in High Glucose DMEM (Sigma Cat. No. D6546), 10% FBS (Cat. No. ES-009-B), 1X L-Glutamine (Cat. No. TMS-002-C) and 1X Penicillin-Streptomycin Solution (Cat. No. TMS-AB2-C) (optional).

2. Remove the vial of frozen IMG 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 IMG 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 10-15 mL of IMG 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 IMG 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.

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Subculturing Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of IMG 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 IMG 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 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 mL of IMG 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 1:5 – 1:7).

Cryopreservation of Cells

IMG Mouse Microglial Cell Line may be frozen in the expansion medium plus 8-10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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