ImKC Murine Immortalized Kupffer Cell Line

Immortalized Cell Line

Cat. # SCC119

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.
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS

Data Sheet

Background

Kupffer cells (KC), also known as stellate macrophages, are liver macrophages that reside in the lumen of the liver sinusoids. Kupffer cells play a critical role in maintaining liver functions including the removal of pathogens & foreign materials, antigen presentation and modulating innate immune responses. *In vitro* studies of Kupffer cells have been hindered by the difficulty in isolating sufficient number of primary cells and the limited lifespan of the cells in culture.

ImKC is a cytokine-producing immortalized Kupffer cell line that was established from transgenic mice expressing the thermolabile mutant tsA58 of the Simian virus 40 large T antigen under the control of the H-2kb promoter. Primary KC were obtained using a three step procedure: liver perfusion, centrifugal elutriation, and sorting for F4/80+ cells. ImKC were identified within the small-intermediate population of KC that maintained stable expression of F4/80, and the surface antigens CD11b, CD14 and TLR4.

ImKC cells are very small and proliferate rapidly. Typically a 1:6 split will be confluent by day 2-3.

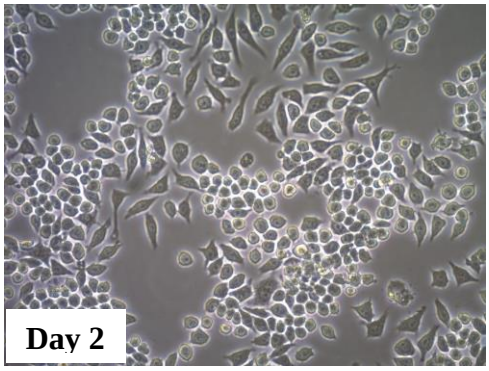
Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Mouse/Rat Comprehensive CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Storage and Handling

ImKC Murine Immortalized Kupffer 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.

Data



References

1. Wang Z, Burlak C, Klaunig JE, and Kamendulis LM (2014) Development of a cytokine-producing immortalized murine Kupffer cell line. *Cytokine* 70(2): 165-172.

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

Please visit www.millipore.com for additional product information and references.

Ver 2.0/2018-02-13/SCC119/AN

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 the following ImKC Expansion Medium:

   - RPMI-1640 (Sigma Cat. No. R8758)
   - 10% FBS (EMD Millipore Cat. No. ES-009-B)
   - 2 mM L-Glutamine (EMD Millipore Cat. No. TMS-002-C)
   - 1X Penicillin-Streptomycin Solution (EMD Millipore Cat. No. TMS-AB2-C)

2. Remove the vial of frozen ImKC 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 ImKC 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 ImKC 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 ImKC Expansion Medium. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 90-95% confluent, they can be dissociated with Accutase (EMD Millipore Cat. No. SCR005) or trypsin-EDTA (EMD Millipore 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 ImKC 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 ImKC 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 ImKC 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

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

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