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cell based solutions

IMMORTALIZED CELL LINES

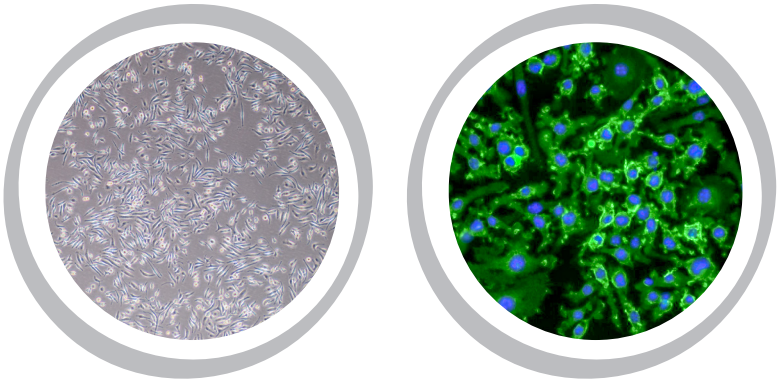
P10626-IM

Mouse Immortalized Kupffer Cells are specialized liver-resident macrophages derived from the mononuclear phagocyte system. These cells play a critical role in liver homeostasis, innate immunity, and the clearance of pathogens, cell debris, and toxins. Due to their phagocytic activity, cytokine production, and interaction with hepatocytes and other immune cells, Kupffer cells are essential for studying liver inflammation, fibrosis, and host- pathogen interactions. Immortalized Kupffer cells provide a reproducible and robust in vitro model to investigate hepatic immune responses, macrophage polarization, and drug metabolism in a murine context.

Mouse Kupffer Cells were immortalized through lentiviral transduction using vectors expressing SV40 large T antigen. Compared to primary mouse Kupffer cells, which exhibit limited lifespan and are difficult to expand, the immortalized cells displayed sustained proliferation over multiple passages while maintaining characteristic macrophage markers and functional properties, enabling long-term experimental applications.

IMMORTALIZED MOUSE KUPFFER CELLS

Product Type: Immortalized Mouse Kupffer Cells
Catalog Number: P10626-IM
Immortalization: SV40 Large T Antigen, G418 resistant.
Number of cells: >1x10^6 cells (cryopreserved vials)
Storage: Liquid Nitrogen
Recommended Medium: Macrophage Medium Kit (Ref: P60136)
Product Characterization: Positive for F4/80.



Brightfield F4/80

About Immortalized Mouse Kupffer cells

Immortalized Mouse Kupffer Cells were generated by transducing primary cells with lentiviral vectors expressing the SV40 large T antigen (Lenti-SV40). Immortalized cells were cultured in parallel with primary cells to assess their proliferation capacity. While primary cells underwent replicative senescence after a few passages, the transduced cells exhibited stable and long-term proliferation, having been expanded beyond 30 passages without signs of growth arrest. The SV40 large T antigen promotes immortalization by inactivating tumor suppressor proteins such as p53 and Rb, ensuring continuous cell proliferation. The resulting immortalized cells maintain consistent morphology, a stable proliferative phenotype, and are suitable for long-term in vitro applications, providing a valuable resource for further studies in regenerative medicine and tissue engineering.

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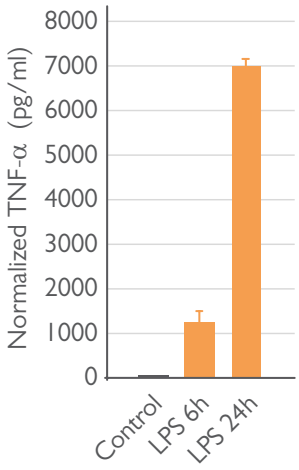
Functional Validation of Kupffer Cell Activation by LPS

Immortalized mouse Kupffer cells were seeded in 96-well plates at a density of 60,000 cells per well in 150 µL of complete medium. Later the same day, the medium was replaced with serum-free basal medium and cells were incubated overnight. On Day 2, cells were stimulated with 500 ng/mL of lipopolysaccharide (LPS) in 150 µL of basal medium for 6 hours. After incubation, the conditioned media were collected and murine TNF-α levels were quantified using an R&D Systems ELISA kit. While basal conditions yielded virtually undetectable TNF-α levels, LPS stimulation induced a robust response.



Culturing conditions

1 IMMEDIATELY UPON DELIVERY

1.1 Remove the vial from the shipping container to check for freezing.

1.2 Transfer the frozen vial to liquid nitrogen until ready to thaw.

2 THAWING CELLS:

2.1 Prepare a fibronectin coated flask (2 µg/cm2, T-75 flask is recommended). Add 10 ml of sterile Dulbecco's phosphate buffered saline (DPBS) to a T-75 flask and then add 150 µl of fibronectin stock solution (1 mg/ml, Innoprot cat. no. P8248). Leave the flask in incubator overnight.

2.2 Prepare "Thawing medium" by combining 500 ml of basal medium, 25 ml of fetal bovine serum, 5 ml of Growth supplement and 5 ml of penicillin/streptomycin solution.

2.3 Thaw cells rapidly in a 37ºC water bath; avoid allowing the sample to warm to 37ºC. Cryovials should be cool to the touch when removed.

2.4 Remove the vial, wipe it dry, and transfer it to a sterile field.

2.5 Rinse the vial with 70% ethanol, then wipe to remove excess. Open the vial and resuspend its contents using a 1 ml Eppendorf pipette.

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Culturing conditions

2.6 Dispense the contents into a 25 cm^2 culture flask with warm complete media (FBS percentage can be increased up to 10% for better culture establishment).

2.7 Place the flask in the incubator.

2.8 For optimal results, avoid disturbing the culture for 16 hours after initiation. Change the growth medium the next day to remove unattached cells, then every other day thereafter.

3 MAINTENANCE OF THE CULTURE:

3.1 Change medium 48 hours after establishing a subculture.

3.2 Subculture when cells are over 90% confluent.

4 SUBCULTURING:

Remove medium, rinse with 0.05% trypsin-EDTA solution. Add 1 to 2 mL of trypsin-EDTA solution and allow the flask to sit until cells detach. Add fresh culture medium, aspirate, and dispense into new culture flasks.

Recommended subcultivation ratio of 1:2 to 1:6.

Medium Renewal: 2 to 3 times per week.

Reagents for cryopreservation: Cryostor S10.

Quality Control / Biosafety

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi.

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