

HMC3 Cells | 300102**General information****Description**

The Human Microglial Clone 3 (HMC3) cell line was developed in 1995 by Professor Tardieu's team through the SV40-dependent immortalization of microglial cells from human spinal cord and cortical tissues, obtained from embryos aged between 8 to 12 weeks. These primary cells, characterized by slow division and complex morphologies, were initially cultured for 10-15 days before immortalization. The HMC3 cells maintained several key features of primary microglia, such as a diverse expression of myeloid markers like CD68, CD11b, and CD14, though the expression levels varied notably with the choice of primary antibody, particularly for CD68.

Following immortalization, the HMC3 cells exhibited enhanced proliferation rates, with doubling times between 24 and 48 hours, while preserving many phenotypic and morphological characteristics of their primary counterparts. Notably, there was a higher proportion of CD68 EBM/11-positive cells and a reduction in phagocytic activity compared to the primary cells. Stability in antigenic expression was confirmed across 35 passages, with the cells remaining positive for NSE, CD68, and CD11b, but negative for CD14, MHCII, and CD4 under baseline conditions. However, exposure to interferon- γ (IFN γ) elevated MHCII expression, aligning more closely with primary culture responses to the same treatment.

Functionally, the HMC3 line distinguished itself by producing higher levels of interleukin-6 (IL-6) under basal conditions compared to other clones. Despite this, a direct comparison with primary microglial cells' cytokine production remains challenging due to methodological differences. The response to lipopolysaccharide (LPS) stimulation in these immortalized lines appeared diminished relative to primary cultures. Consistent with primary microglial characteristics, the HMC3 and other cloned lines did not produce tumor necrosis factor-alpha (TNF α), either spontaneously or following pro-inflammatory stimulation, highlighting a specific trait of human embryonic microglia.

Organism Human**Tissue** Fetal brain**Applications** 3D cell culture, Neuroscience, Neuroinflammation**Synonyms** Human Microglia Clone 3, CHME-3, CHME3**Characteristics****Age** Fetus**Gender** Unspecified**Morphology** Macrophage**Cell type** Microglial cell

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Growth properties	Adherent
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Identifiers / Biosafety / Citation

Citation	HMC3 (Cytion catalog number 300651)
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Biosafety level	1
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Expression / Mutation

Viruses	The SV40 genetic material is stably integrated into the cell genome. There is no active production or release of complete viral particles, which mitigates potential biosafety concerns.
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Handling

Culture Medium	DMEM:Ham's F12, w: 3.1 g/L Glucose, w: 1.6 mM L-Glutamine, w: 15 mM HEPES, w: 1.0 mM Sodium pyruvate, w: 1.2 g/L NaHCO ₃ (Cytion article number 820400a)
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Medium supplements	Supplement the medium with 10% FBS
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Passaging solution	Accutase
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Doubling time	24 and 48 hours
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Subculturing	Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.
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Freeze medium	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)
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Handling of cryopreserved cultures

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium. Optionally, skip centrifugation but remove any remaining freezing medium after 24 hours.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

Quality control / Genetic profile / HLA

Sterility

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.

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STR profile

Amelogenin: x,x
CSF1PO: 10,11
D13S317: 11
D16S539: 12,13
D5S818: 11,12
D7S820: 9,11
TH01: 6
TPOX: 8,9
vWA: 17,19
D3S1358: 16,18
D21S11: 30,31.2
D18S51: 18
Penta E: 7,13
Penta D: 10,14
D8S1179: 13,14
FGA: 21,25
D6S1043: 11
D2S1338: 17,25
D12S391: 16,21
D19S433: 15,15.2