HMC3

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 (IFNγ) 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 (TNFα), either spontaneously or following pro-inflammatory stimulation, highlighting a specific trait of human embryonic microglia.

Tissue	Fetal brain

Applications

3D cell culture, Neuroscience, Neuroinflamation

Synonyms

- Human Microglia Clone 3
- CHME-3
- CHME3

CLASSIFICATION

Organism	Human
Sex	Unspecified
Growth properties	Adherent
GMO status	GMO-S1

GMO status details

GMO-S1: This human fetal brain microglia cell line (HMC3) contains an SV40 T-Antigen construct introduced by transfection, supporting immortalization. The insert is stably present in microglia-derived cells. This classification applies only within Germany and may differ elsewhere.

Biosafety level	1

CHARACTERISTICS

Morphology	Macrophage

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Cell type	Microglial cell
Doubling time	24 and 48 hours

IDENTIFIERS

Citation

HMC3 (Cytion catalog number 300102)

NCBI TaxID	9606
Cellosaurus	CVCL_II76

EXPRESSION AND SUSCEPTIBILITY

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.

HANDLING AND CULTURE

Culture medium

DMEM:Ham's F12 (1:1), w: 3.1 g/L Glucose, w: 2.5 mM L-Glutamine, w: 15 mM HEPES, w: 0.5 mM Sodium pyruvate, w: 1.2 g/L NaHCO3 (Cytion article number 820400a)

Incubation atmosphere	37°C, 5% CO_2, humidified atmosphere.

Supplements

Supplement the medium with 10% FBS

Dissociation reagent	Accutase

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.

Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
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.
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.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
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.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
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.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

QUALITY CONTROL AND CRYOPRESERVATION

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.

Freeze medium	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

Storage conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Shipping conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

DONOR

Donor age	Fetus

LEGAL AND SAFETY

Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

Material Transfer Agreement required	Yes

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