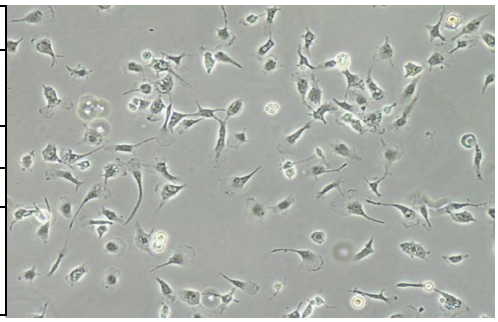
NEUROMICS

Immortalized Human Brain Microglia Cells

Catalog Number	HBMCs001
Product Name	**Immortalized Human Brain Microglia Cells**
Storage	Liquid Nitrogen
Product Format	Frozen vial
Cells Number	500,000 cells



***Caution:** The handling of human derived products has the potential to be biologically hazardous. All Cell strains tested negative for HIV, HBV, and HCV DNA in diagnostic tests. Proper precautions must be taken to avoid exposure. Always wear proper protective equipment (Gloves, safety glasses, etc.) when handling these materials. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.

GENERAL INFORMATION:

Microglia, one of the glial cell types in the CNS, is an important integral component of neuroglia cell network. They have been observed in the brain parenchyma from the early stage of development to the mature state. Microglia act as brain macrophages when programmed cell death occurs during brain development or when the CNS is injured or pathologically damaged.

Microglia can be considered as the main cell in brain immune surveillance, can present antigens in the molecular context of MHC class II expression to CD-4 positive T cells, are capable of Fc mediated phagocytosis, and share many common antigens with hemopoietic and tissue macrophages. Furthermore, there is accumulating evidence that microglia are involved in a variety of physiological and pathological processes in the brain by interacting with neurons and other glial cells and through production of biologically active substances such as growth factors, cytokines, and other factors.

Human brain microglia cells are isolated from healthy human brain tissue. After purification, HBMCs are cryopreserved and delivered frozen. HBMCs are ready to plate in a culture vessel for experiment, and can be expanded or long-term cultured. It is recommended to use Microglia Cell Medium for the culturing of HBMCs.

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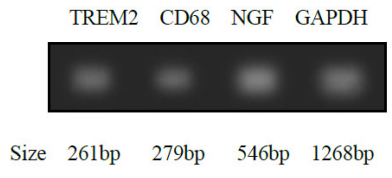
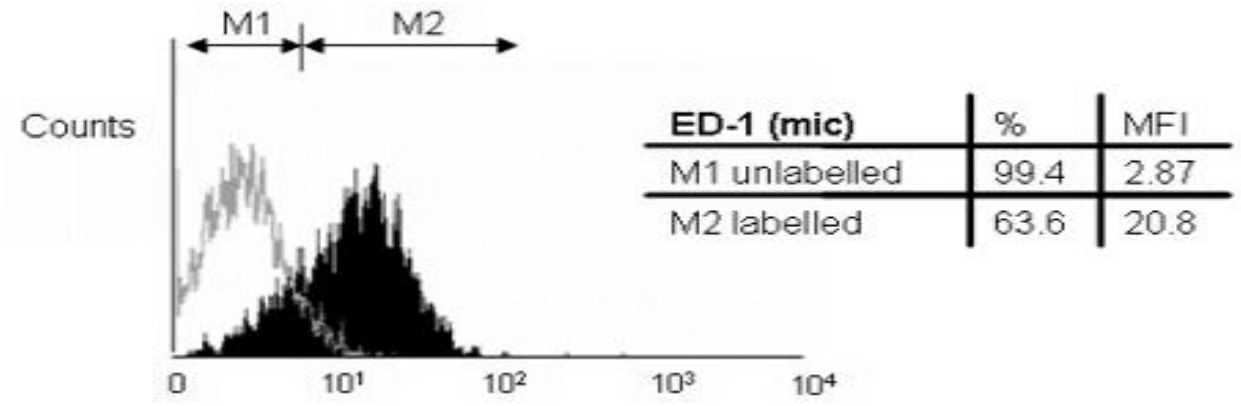
Characterization of the cells:

Gene	Cell Type	Specification	Results	
TREM2	Microglial	Positive	Pass	
CD68	Macrophage	Positive	Pass	
NGF	Neurotrophic	Positive		
GAPDH	House-Keeping	Positive	Pass	



ED-1 (mic)	%	MFI
M1 unlabelled	99.4	2.87
M2 labelled	63.6	20.8

Microglia were isolated and left in culture for 24 hours. The cells were subsequently harvested, fixed then analyzed by flow cytometry using anti-CD68 (ED-1) antibodies. Labeled cells are represented by the black shaded populations, whereas the unlabeled cells are depicted by the grey line (%: % of cells in M1 or M2 region, MFI: mean fluorescence intensity).

Cytoplasmic F4/80: **>98% positive by immunofluorescence**
CD68: **>98% positive by immunofluorescence**

The cells maintained microglial specific markers such as NGF, CD68 and TREM2 as demonstrated by RT-PCR. These cells are suitable for studies of human microglia in health and disease.

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Handling of Arriving Cells:

When you receive the cells in a frozen vial, you can transfer the vial of cells into a -80ºC freezer for short period storage or a liquid nitrogen tank for long term storage. Thaw the cells in a 37°C water bath, and then transfer the cells into a T25 flask pre-coated with poly-L-lysine as described in details in Subculture Protocol.

Thawing Protocol:

1. Coat flask with:
    a. Poly-L-lysine
        i. Prepare a poly-L-lysine coated flask (2µg/cm^2 , T-25 flask is recommended). Add 5 ml of sterile water to a T-25 flask and then add 9µl of poly-L-lysine stock solution 10mg/ml. leave the flask in incubator overnight (minimum one hour at37°C incubator).
        ii. Rinse the poly-L-lysine coated flask with sterile water twice and add 7 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.

    OR

    b. AlphabioCoat Solution (AC001)
        i. Add 2 ml AlphaBioCoat Solution (AC001) into a T25 flask and ensure entire interior surface is coated with solution. After 30 minutes, dispose of alphabiocoat by aspiration. Gently rinse and aspirate flask with phosphate buffer solution (PBS300). The flask is now ready for use(no need for overnight incubation when coated with AC001)
        ii. If you are using the coated flask the same day, add about 4 ml of Microglial media to the coated flask. *If the media changes color from pink to yellow, aspirate and discard the media. Add 4ml of fresh media to the coated flask.
2. Warm MCM before thawing the cells.
3. Place the vial in a 37°C water bath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the water bath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml Eppendorf pipette gently resuspend the contents of the vial.
4. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of ≥10,000 cells/cm^2 is recommended. ***Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that cells are plated in poly-L-lysine coated culture vessels that promote cell attachment.***

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5. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
6. Return the culture vessels to the incubator.
7. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every two to three days thereafter.

Subculture Protocol

1. When flask is at 90% confluence, aspirate media from flask.
2. Rinse T25 flask containing cells with 5 ml 1XPBS (cat#PBS300).
3. Gently aspirate out the PBS after rinsing, and discard.
4. Add 2ml of RT trypsin/ EDTA (0.5%) or Cell Detachement Solution (ADF001) to T25 flask containing cells (ensure entire interior surface is cover).
5. Place T25 flask containing cells into 37°C incubator for 1 or 2 minutes (cells will normally come off from the surface within 1 or 2 minutes).
6. Suspend the cells with 15ml of AlphaGlia Media (AGEM-001) and transfer equally into 3 pre-coated T25 flasks (coating procedure described previously) (the cells are now at a subculture ratio of 1:3).
7. There is no need to spin cells during subculture.
8. Proliferating cell culture: AlphaGlia Media (AGEM-001) should be changed every 2 days. The cells normally become confluent within 7 days (when split at a 1:3 ratio)

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