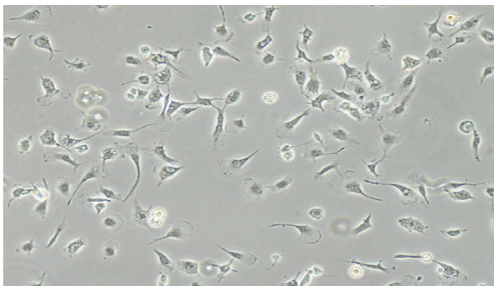
Catalog Number	PHM001
Product Name	**Primary Human Brain Microglia Cells**
Storage	Liquid Nitrogen
Product Format	Frozen vial
Cells Number	>90% confluent in Frozen Vial 500.000 cells



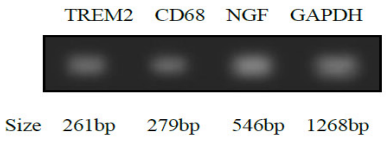
*Caution: The handling of human derived products has the potential to be biologically hazardous. All Cell strains tested negative for HIV, HBV, and HCV DNA in diagnostic tests. Proper precautions must be taken to avoid exposure. Always wear proper protective equipment (Gloves, safety glasses, etc.) when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

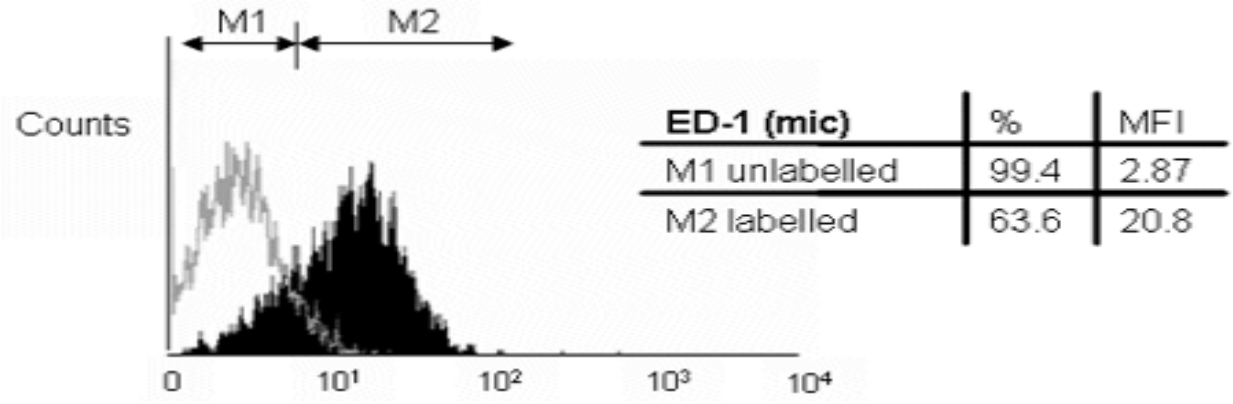
GENERAL INFORMATION:

Microglia, one of the glial cell types in the CNS, is an important integral component of neuroglial 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, Cat.#PHM001 are cryopreserved and delivered frozen. Cat.#PHM001 are ready to plate in a culture vessel for experiment, but not recommended for expanding or long term cultures since the cells do not proliferate in culture. It is recommended to use Alpha-Glia Growth and Expansion Medium Cat.#AGEM001 for the culturing of Cat.#PHM001.

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.

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 Cat.# AC001 AlphaBioCoat Coating Solution as described in details in Subculture Protocol.

Subculture Protocol:

*All cryovials should be stored in liquid nitrogen immediately upon arrival, unless thawed for cells culture.

Frozen Vial:

1) Coating T25 flasks. Add 2 ml AlphaBioCoat (Cat.#AC001) into a T25 flask and ensure entire interior surface is coated with the solution. After 30 minutes, dispose of AlphaBioCoat (Cat.#AC001) by aspiration. Gently rinse and aspirate flask with Phosphate Buffer Solution (Cat.#1XPBS-001). The flask is now ready for use (no need for overnight incubation when coated with Cat.#AC001).
2) If you are using the coated flask the same day, add about 4 ml of Alpha-Glia cells Growth and Expansion media Cat.#AGEM001 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.
3) Thaw the cells in a 37°C water bath. Once you see a small amount of ice left in the vial, spray the vial with 70% Ethanol and wipe it down.
4) Transfer the vial into your Biosafety cabinet.
5) Using a 2 or 5ml pipet, pipet the cells out of the vial.
6) Transfer your cell suspension in to your coated plate that have the 4 ml media in it.
7) You should have a total working volume of 5ml of cell suspension in the flask; close the cap. Make sure cells are evenly distributed in the flask by moving the flask left and right five times. Move it up and down for an additional five times.
8) Place flask in a 37°C incubator with 5% C02. If flask is not vented, please loosen cap.
9) Change media after 48 hours.
10) For assay or to induce quiescent cells, use Alpha-Glia Basal Media Cat.#AGEM002
11) End of protocol