Human Microglia (HM)

Catalog #1900

Cell Specification

Microglia, one of the glial cell types in the central nervous system (CNS), is an important integral component of the neuro-glial cell network [1]. They are present in the brain parenchyma from the early stage of development to the mature state. Microglia play an important role in brain immune surveillance. They can present antigens in the molecular context of MHC class II expression to CD-4 positive T cells, and are capable of Fc-mediated phagocytosis, and share many common antigens with hemopoietic and tissue macrophages [2]. Upon activation, they act as brain macrophages to clear tissue debris, damaged cells, or microbes when programmed cell death occurs during brain development or when the CNS is injured. Furthermore, there is evidence that microglia are involved in a variety of physiological and pathological processes in the brain through interaction with neurons, other glial cells, and production of biologically active substances such as growth factors and cytokines[3].

HM from ScienCell Research Laboratories are isolated from human brain. HM are cryopreserved after purification and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. HM are characterized by immunofluorescence with antibodies to CD 11b and F4/80. HM are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. HM are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; however, *HM are not recommended for expanding or long-term cultures since the cells do not proliferate in regular culture.*

Recommended Medium

It is recommended to use Microglia Medium (MM, Cat. #1901) for culturing HM *in vitro*.

Product Use

HM are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Lee SC, Liu W, Brosnan CF, Dickson DW. (1992) "Characterization of primary human fetal dissociated central nervous system cultures with an emphasis on microglia." *Laboratory Investigation*. 67: 465-76.

[2] Fedoroff S, Zhai R, Novak JP. (1997) "Microglia and astroglia have a common progenitor cell." *J Neurosci Res*. 50: 477-86.

[3] Stoll G, Jander S. (1999) "The role of microglia and macrophages in the pathophysiology of the CNS." *Prog Neurobiol*. 58: 233-47.

Instructions for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C water bath and return the cells to culture as quickly as possible with minimal handling!

Note: *Experiments should be well organized before thawing HM. It is recommended that HM are used for experiments as quickly as possible after thawing the cells.* ***HM cannot be subcultured or passaged, as the cells do not proliferate.***

Initiating the culture:

1. Prepare a poly-L-lysine-coated culture plate (2 μg/cm^2 is recommended). For example, add 2 ml of sterile water to one well of a 6-well plate and then add 20μl of poly-L-lysine stock solution (1 mg/ml, Cat. #0403). Leave the plate in a 37°C incubator overnight (or for a minimum of one hour).
2. Prepare complete medium (MM, Cat. #1901). Thaw MGS (Cat. #1952), FBS (Cat. #0025) and P/S solution (Cat. #0503) at 37°C. Gently tilt the tubes several times to ensure the contents are completely mixed before adding to the medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. In a sterile field, remove the caps without touching the interior threads with fingers. Add MGS, FBS and P/S solution to the medium and mix well.
3. Rinse the poly-L-lysine-coated culture vessel twice with sterile water and then add the volume of complete medium recommended in Table 1 or Table 2. Leave the plate(s) in the sterile field and proceed to thaw the cryopreserved cells.
4. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.

   Note*: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in poly-L-lysine-coated culture vessels to promote cell attachment.*
5. Carefully remove the cap without touching the interior threads and gently resuspend the cell suspension. A seeding density of 10,000-20,000 cells/cm^2 is recommended depending on your experiments. We recommend following Table 1 for seeding HM onto 6-well, 12-well, or 24-well plates. For seeding HM on 60 mm plates, use Table 2.

Table 1
Recommended cell suspension volume per vial using a 6-well, 12-well, or 24 well format

Well format	Surface area/well (approx. values)	Volume of media/well	Volume of cell suspension from vial/well	# of wells/vial
6-well	9.6 cm^2	3.0 ml	150 μl	6 wells
12-well	3.9 cm^2	2.0 ml	60 μl	15 wells
24-well	1.9 cm^2	1.0 ml	30 μl	30 wells

Table 2
Recommended cell suspension volume per vial using 60 mm plates

Plate Format	Surface area/plate (approx. values)	Volume of cell suspension from vial/plate	# of plates/vial	Volume of media (ml)/plate
60 mm	21 cm^2	300 µl	3	3.0 ml

6. Pipet the correct volume of cell suspension into each well of an equilibrated, poly-$_L$-lysine-coated culture plate containing complete medium. Replace the lid of the culture plate and gently rock the plate to distribute the cells evenly.
7. Return the culture plate to the incubator.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the culture medium in 24 hrs to remove residual DMSO and unattached cells. Once the microglia attach and spread, the cells can be used for experiments.

***Note**: Cells may take a few days to attach and spread.*

9. Use cells promptly for experiments.

***Caution**: Handling human derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].*

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods* 11:191-9