Rat Microglia
(RM)

Catalog Number: R1900

Cell Specification

Microglia, one of the glial cell types in the CNS, is an important integral component of the neuro-glial cell network [1]. 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 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 [2]. 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 [3].

RM from ScienCell Research Laboratories is isolated from primary rat brain cell culture. Cells are harvested after purification and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. RM is characterized by immunofluorescent method with antibody to OX-42 (CD 11b/c). RM is negative mycoplasma, bacteria, yeast and fungi. RM is guaranteed to further culture in the conditions provided by ScienCell Research Laboratories.

Product Use

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

Storage

Transfer cells directly and immediately from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.

Shipping

Dry ice or gel ice.

Reference

[1] Lee, S. C., Liu, W., Brosnan, C. F. and Dickson, D. W. (1992) Characterization of primary human fetal dissociated central nervous system cultures with an emphasis on microgia. Laboratory Investigation. 67:465-476.

[2] Fedoroff, S., Zhai, R. and Novak, J. P. (1997) Microglia and astroglia have a common progenitor cell. J. Neurosci. Res. 50: 477-486.

[3] Stoll, G. and Jander, S. (1999) The role of microglia and macrophages in the pathophysiology of the CNS. Prog. Neurobiol. 58:233-247.

Instruction for culturing cells

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

Set up culture after receiving the ordering:

1. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, and transfer it to a sterile field. Rinse the vial with 70% ethanol, and then wipe to remove excess. 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.
2. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 10,000 cells/cm^2 (a T-45 flask) 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 microglia is plated in poly-L-lysine coated culture vessels that promote the cell attachment.
3. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
4. Return the culture vessels to the incubator.
5. 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. A health culture of microglia show characteristic elongated, almost bipolar cell bodies with spine-like processes that often branch perpendicularly.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture.
2. Change the medium every other day thereafter, until the culture is approximately 50% confluent.
3. Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent.

Subculture:

Microglia is not supposed to be subcultured since this cell type is terminally differentiated cells. The following is only for reference in case you need to subculture them.

1. Prepare poly-L-lysine coated cell culture flasks.
2. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to **room temperature**. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.
3. Rinse the cells with DPBS.
4. Incubate cells with 3 ml of trypsin/EDTA solution (in the case of T-25 flask) until 80% of cells are rounded up (monitored with microscope). Add 3 ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel.
   Note: Use ScienCell Research Laboratories' trypsin/EDTA solution that is optimized to minimize the killing of the cells by over trypsinization.
5. Harvest and transfer released cells into a 15 ml centrifuge tube. Rinse the flask with another 3 ml of growth medium to collect the residue cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%.
6. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.
7. Count cells and plate them in a new, poly-L-lysine coated flask with cell density as recommended.

Caution: Handling animal derived products is potentially bioharzadous. Although each cell strain testes negative for microbial, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working 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, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11(4).

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