



**Rat Microglia
(RM)**
Catalog #R1900

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 have been observed 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].

RM from ScienCell Research Laboratories are isolated from postnatal day 2 CD® IGS rat brain. RM are cryopreserved at P0 and delivered frozen. Each vial contains $>1 \times 10^6$ cells in 1 ml volume. RM are characterized by immunofluorescence with antibody to F4/80. RM are negative for mycoplasma, bacteria, yeast, and fungi. RM are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; however, *RM 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 RM *in vitro*.

Product Use

RM 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 primary 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! Do not centrifuge the cells after thawing as this can damage the cells.

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

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37°C, 5% CO₂ incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a poly-L-lysine-coated culture plate (2 µg/cm² 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 MGS tube several times to ensure the contents are completely dissolved 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 cap, being careful not to touch 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/cm² is recommended depending on your experiments. We recommend following Table 1 for seeding RM onto 6-well, 12-well, or 24-well plates. For seeding RM 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 microglia attach, the culture is ready for experiment.

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

9. Once cells are attached use promptly for experiments.

Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

Caution: Handling animal-derived products is potentially biohazardous. 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