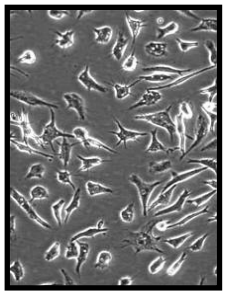
REF: P10304

NEUROSCIENCES INNOPROFILE™

RAT MICROGLIA



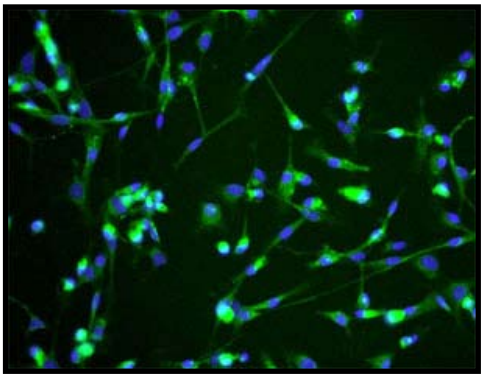
Product Type:	Cryo-preserved Microglia
Catalog Number:	P10304
Source:	Rat Brain
Number of cells:	5 x 10^5 cells / vial (1ml)
Storage:	Liquid Nitrogen

Rat Microglia (RM) provided by Innoprot are isolated by Innoprot from primary rat brain cell culture. RM are harvested after purification and delivered frozen. RM is guaranteed to further culture in the conditions provided by Innoprot.

Microglia, one of the glial cell types in the CNS, are an important integral component of neuro-glial cell network. Microglia act as brain macrophages when programmed cell death occurs during brain development or when the CNS is injured. 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.

Recommended Medium

* Ref: P60116: Microglia Medium



Product Characterization

Immunofluorescent method

- Antibody to F4/80

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi

Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures

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INSTRUCTIONS FOR CULTURING CELLS

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

Set up culture after receiving the order:

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. #PLL). Leave the plate in a 37°C incubator overnight (or for a minimum of one hour).

2. Prepare complete medium. Thaw MGS, FBS and P/S solution 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/cm^2 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.

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.

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

9. Use cells promptly for experiments. 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]

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).

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

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