Rat Oligodendrocyte Precursor Cells (ROPC)

Catalog #R1600

Cell Specification

The precursor cells of oligodendrocytes, the myelin-forming cells of the central nervous system, were first discovered in 1993 by Raff, Miller, and Noble [1]. They have been extensively studied and are referred to in the literature as either oligodendrocyte-type-2 astrocyte progenitor cells or oligodendrocyte precursor cells (OPC). The developing and adult central nervous system both contain OPC [2, 3]. In culture, OPC can be generated from neural progenitors or neural stem cells by the stimulation of basic fibroblast growth factor. OPC proliferate in the presence of platelet-derived growth factor or factors produced by astrocytes, and differentiate into mature oligodendrocytes. Based on these qualities, OPC provide an exceptional model to study developmental transitions.

ROPC from ScienCell Research Laboratories are isolated from postnatal day 2 CD® IGS rat brain. ROPC are cryopreserved after purification and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. ROPC are characterized by immunofluorescence with antibodies specific to A2B5 and O4. ROPC are negative for mycoplasma, bacteria, yeast, and fungi. ROPC are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; however, *ROPC are not recommended for expanding or long-term cultures due to limited expansion capacity*.

Recommended Medium

It is recommended to use Oligodendrocyte Precursor Cell Medium (OPCM, Cat. #1601) for culturing ROPC *in vitro* and Oligodendrocyte Precursor Cell Differentiation Medium (OPCDM, Cat. #1631) for differentiating ROPC.

Product Use

ROPC 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] Raff MC, Miller RH, Noble M. (1983) "A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on the culture medium." *Nature*. 303: 390-6.

[2] ffrench-Constant C, Raff MC. (1986) "Proliferating bipotential glial progenitor cells in adult rat optic nerve." *Nature*. 319: 499-502.

[3] Wolswijk G, Noble M. (1989) "Identification of an adult-specific glial progenitor cell." *Development*. 105: 387-400.

[4] Noble M, Murray K, Stroobant P, Waterfield MD, Riddle P. (1988) "Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cells." *Nature*. 333: 560-2.

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 ROPC. It is recommended that ROPC are used for experiments as quickly as possible after thawing the cells.* ***ROPC 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 (OPCM, Cat. #1601). Thaw OPCGS (Cat. #1652), 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 OPCGS 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 ROPC onto 6-well, 12-well, or 24-well plates. For seeding ROPC 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.

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

9. Use cells promptly for experiments once cells attach and spread.

***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

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