Rat Oligodendrocyte Precursor Cell (ROPC)

Catalog Number: R1600

Cell Specification

The precursor cells for oligodendrocytes were first discovered in 1993 by Raff, Miller and Noble [1] and have been extensively studied. These precursor cells are referred 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]. Oligodendrocytes, the myelin-forming cells of the central nervous system, develop from OPC. In culture, OPC can be generated from neural progenitors or neural stem cells in the presence of basic fibroblast growth factor and they proliferate in presence to platelet-derived growth factor or factors produced by astrocytes [4] and differentiate into mature oligodendrocytes. Because of this, they have provided an exceptional population in which to study developmental transitions.

ROPC from ScienCell Research Laboratories are isolated from neonate day 2 rat brain tissues. ROPC are cryopreserved immediately after purification and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. ROPC are characterized by immunofluorescent method with antibodies to A2B5 and nestin. ROPC are negative for mycoplasma, bacteria, yeast and fungi. ROPC are guaranteed to further culture at the conditions provided by ScienCell Research Laboratories.

Product Use

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

Storage

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

Shipping

Dry ice.

Reference

[1] Raff, M. C., Miller, R. H. and Noble, M. (1983) A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on the culture medium. Nature 303:390-396.

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

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

[4] Noble, M., Murray, K., Stroobant, P., Waterfield, M. D. Riddle, P. (1988) Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cells. Nature 333:560-562.

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!

Unpacking

1. For crypreserved cells: If there is dry ice in the package and you are not going to culture cells right way, place cryovial(s) immediately into liquid nitrogen. If there is no dry ice left in the package, thaw and culture the cells immediately.

2. For proliferating cells: Spray the culture vessel (flask, plate or slide) with 70% ethanol for disinfection. Transfer the cells into 37°C, 5% CO_2 incubator and allow equilibrating for 2 hours. After cells have equilibrated, remove shipping medium from the culture vessel and replace with fresh medium.

Set up culture after receiving the ordering

1. Coat culture vessel with laminin or poly-L-lysine.
   Note: It is important that oligodendrocyte precursor cells are plated in laminin or poly-L-lysine coated culture vessels that promote cell attachment and neurites outgrowth (poly-L-lysine coating: coat flask or plate with poly-L-lysine at 2 μg/ml concentration for one hour and wash the flask or plate with sterile water three times).

2. Medium preparation: Decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with the medium to recover the entire volume.

3. Set up culture: Prepare one T-45 flask for each cryovial. Add the appropriate amount of medium to the vessel (recommend for 15 ml/T-45 flask) and allow the flask to equilibrate in 37°C, 5% CO_2 incubator for at least 30 min.

4. Thawing of cells: 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.

5. Using 1 ml eppendorf pipette gently resuspend the cells in the vial and transfer them to equilibrated culture vessels (a T-45 flask). A high seeding density (>20,000/cm^2) 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.

6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange. Return the culture vessels to the incubator.

7. 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 will display normal oligodendrocyte precursor cell morphology (unipolar or dipolar processes with small and round phase-bright cell body).

Subculture:

1. Subculture the cells when they are 90% confluent.
2. Prepare laminin or poly-L-lysine-coated cell culture flasks.
3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to room temperature.
4. Rinse the cells with DPBS.
5. Incubate cells with 5 ml of trypsin/EDTA solution (in the case of T-45 flask) until 80% of cells are rounded up (monitored with microscope). Add 3 ml of trypsin neutralization solution to the digest 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.
6. 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%.
7. Centrifuge the cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.
8. 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. 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).