



## Human Perineurial Cells (HPNC)

Catalog Number: 1710

### Cell Specification

Perineurial cells are of mesenchymal origin. They make up the perineurium, which has the important role of maintaining the integrity of the internal peripheral nerve environment by creating a physical barrier that, under physiologic condition, limits the entry of biologically active proteins, infectious agents, and migration of blood-borne cells into the nerve bundles [1]. The perineurial cells are characterized by distinct ultrastructural features, including non-branching thin cytoplasmic processes coated by an external lamina and joined at their ends by a tight junction, few organelles, actin and vimentin filaments, and numerous pinocytotic vesicles [2]. They initially form a loose, permeable sheath around axons and Schwann cells, which may recruit them from the surrounding mesenchyme, and from which they are separated by the extracellular matrix. These cells later undergo a mesenchymal-to-epithelial transition, forming tight junctions and organizing into the perineurium. The perineurial cells are immunoreactive for vimentin and epithelial membrane antigen but not for the Schwann cell markers S100 protein [3].

HPNC from ScienCell Research Laboratories are isolated from human spinal nerves. HPNC are cryopreserved at passage one and delivered frozen. Each vial contains  $>5 \times 10^5$  cells in 1 ml volume. HPNC are characterized by immunofluorescent method with antibodies to Vimentin, S100, GFAP and CD90. HPNC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HPNC are guaranteed to further expand for 15 population doublings in the conditions provided by ScienCell Research Laboratories.

### Recommended Medium

It is recommended to use Fibroblast Medium (FM, Cat. No. 2301) for the culturing of HPNC *in vitro*.

### Product Use

HPNC are 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.

### Reference

- [1] Salzer, J. L. (1999) Creating barriers: a new role for Schwann cells and desert hedgehog. *Neuron* 22:627-629.
- [2] Erlandson, R. A. (1991) The enigmatic perineurial cell and its participation in tumors and in tumor like entities. *Ultrastruct Pathol.* 15:335-351.
- [3] Ariza, A., Bilbao, J. M. and Rosai, J. (1988) Immunohistochemical detection of epithelial membrane antigen in normal and perineurial cells and perineurioma. *Am J Surg Pathol.* 12:678-683.

# Instruction for culturing cells

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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 order:

1. Prepare a poly-L-lysine coated flask ( $2\text{ }\mu\text{g}/\text{cm}^2$ , T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15  $\mu\text{l}$  of poly-L-lysine stock solution (10 mg/ml, ScienCell cat. no. 0413). Leave the flask in incubator overnight (minimum one hour at 37°C incubator).
2. Prepare complete medium: 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 medium to recover the entire volume.
3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. 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, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using a 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of  $5,000\text{ cells}/\text{cm}^2$  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. It is also important that cells are plated in poly-L-lysine coated culture vessels that promote cell attachment.*
6. Replace the cap or cover of flask, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. 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 healthy culture will display stellate or spindle-shaped cell morphology, nongranular cytoplasm, and the cell number will be double after two to three days in culture.

## 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:**

1. Subculture the cells when they are 90% confluent.
2. Prepare poly-L-lysine coated cell culture flasks ( $2 \mu\text{g}/\text{cm}^2$ ).
3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to **room temperature**. We do not recommend warming the reagents and medium at  $37^\circ\text{C}$  waterbath prior to use.
4. Rinse the cells with DPBS.
5. Incubate cells with 10 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope). Add 10 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.*

6. Harvest and transfer released cells into a 50 ml centrifuge tube. Rinse the flask with another 10 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 harvested 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 human derived products is potentially biohazardous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must 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).