Human Leptomeningeal Pericytes (HLP)

Catalog #1410

Cell Specification

Central nervous system pericytes are perivascular cells that are closely associated with the endothelium of capillaries and other small vessels [1]. Pericytes, located near endothelial cells, communicate with other cells by extending long cytoplasmic processes which wrap around the capillaries [1, 2]. Pericytes participate in a variety of processes including angiogenesis, endothelial cell survival, regulation of capillary blood flow, and establishment and maintenance of the blood-brain barrier [3, 4]. Pericyte dysregulation has been linked to several pathological conditions such as hypertension, diabetic retinopathy, atherosclerosis, multiple sclerosis, Alzheimer's disease, and tumor angiogenesis [2, 4]. The unique and diverse functions of pericytes make them novel candidates for cell therapy in regenerative medicine. Leptomeningeal pericytes (LP) are derived from the leptomeningeal layer surrounding the brain. Cultured human leptomeningeal pericytes (HLP) are a useful *in vitro* model for studying a wide variety of central nervous system diseases.

HLP from ScienCell Research Laboratories are isolated from human brain. HLP are cryopreserved at passage one after purification and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. HLP are characterized by immunofluorescence with antibody specific to α-smooth muscle actin and platelet derived growth factor-β (PDGF-β). HLP are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. HLP are guaranteed to further expand for 15 population doublings under the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Pericyte Medium (PM, Cat. #1201) for culturing HLP *in vitro*.

Product Use

HLP 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] Dore-Duffy P, Cleary K. (2011) "Morphology and properties of pericytes." *Methods Mol Biol*. 686:49-68.

[2] Allt G, Lawrenson JG. (2001) "Pericytes: cell biology and pathology." *Cells Tissues Organs*. 169: 1-11.

[3] Daneman R, Zhou L, Kebede A, Barres B. (2010) "Pericytes are required for blood-brain barrier integrity during embryogenesis." *Nature*. 468:562-566.

[4] Kutcher M, Herman I. (2009) "The pericyte: cellular regulator of microvascular blood flow." *Microvasc Res*. 77: 235-246.

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!

Initiating the culture:

1. Prepare a poly-L-lysine-coated culture vessel (2 µg/cm^2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-L-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37°C incubator overnight (or for a minimum of one hour).
2. Prepare complete medium (PM, Cat. #1201). Thaw PGS, FBS and P/S solution at 37°C. Gently tilt the tubes several times to ensure the contents are completely mixed before adding to the medium. Spray the medium bottle and tubes with 70% ethanol, and wipe to remove excess liquid. In a sterile field, remove the caps without touching the interior threads with fingers. Add PGS, FBS and P/S solution to the medium and mix well.
3. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 15 ml of complete medium. Leave the vessel 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.
5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, poly-L-lysine-coated culture vessel. A seeding density of 5,000-7,000 cells/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 residual DMSO in the culture. It is also important that cells are plated in poly-L-lysine-coated culture vessels to promote cell attachment.
6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.
7. Return the culture vessel to the incubator.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells, then every other day thereafter.

Maintaining the Culture:

1. Refresh supplemented culture 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% confluency, change medium every day until the culture is approximately 80% confluent.

Subculturing:

1. Subculture when the culture reaches 90% confluency or above.
2. Prepare poly-$_L$-lysine-coated culture vessels (2 μg/cm^2) one day before subculture.
3. Warm complete medium, trypsin/EDTA solution (T/E, Cat. #0103), T/E neutralization solution (TNS, Cat. #0113), and DPBS (Ca^{++}- and Mg^{++}-free, Cat. #0303) to **room temperature**. We do not recommend warming reagents and medium in a 37°C water bath prior to use.
4. Rinse the cells with DPBS.
5. Add 10 ml of DPBS and then 2 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37°C incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another 1 minute (no solution in the flask at this moment).
8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.
9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5 ml of TNS to collect the residual cells.
10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.

    Note: Use ScienCell T/E solution that is optimized to minimize cell damages due to over trypsinization.
11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.
12. Count and plate cells in a new poly-L-lysine-coated culture vessel with the recommended cell density.

Caution: Handling human derived products is potentially biohazardous. Although each cell strain tests 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 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.