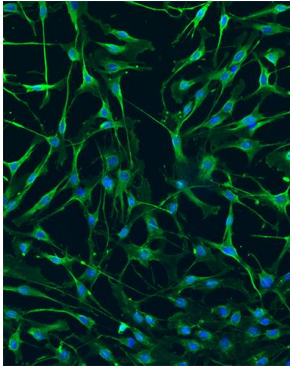


BRAIN CELL SYSTEM INNOPROFILE™

IMMORTALIZED HUMAN BRAIN VASCULAR PERICYTES



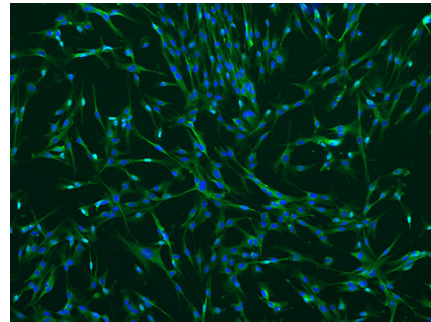
Product Type:	Immortalized Human Brain Vascular Pericytes
Catalog Number:	P10363-IM
Immortalization:	SV40 antigen T
Number of cells:	> 1 x10 ⁶ cells in Cryopreserved vials
Storage:	Liquid Nitrogen

P10363-IM have been obtained immortalizing Human Primary Brain Vascular Pericytes with Lenti-SV40T Lentivirus. Immortalized cells were controlled passaging side by side with the primary cells. Primary cells go into senescence after the 4th passage while the SV40T-transduced cells go beyond 30 passages.

Pericytes are contractile smooth muscle-like cells that cover the albuminal surface of microvessels. They are most abundant on venules and are common on capillaries. Three major functional roles have been ascribed to pericytes associated with central nervous system microvesicular-contractility, regulation of endothelial cell activity, and macrophage activity. There is also evidence that pericytes are involved in the transport across the blood-brain barrier and the regulation of vascular permeability. An important role for pericytes in pathology has been indicated in hypertension, diabetic retinopathy, Alzheimer's disease, multiple sclerosis and central nervous system tumor formation.

Recommended Medium

- Pericyte Medium Kit PLUS
(Reference: P60121-PLUS)



Product Characterization

- Vimentin (IF)
- Smooth Muscle Actin (IF)
- PDGFR-β (IF)

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

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!

Unpacking:

1. For cryopreserved 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.

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 mesangial cells are plated in cell culture vessels that promote cell attachment.

Set up culture after receiving the order:

1. 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.
2. 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 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated culture vessels. A seeding density higher than 7,500 cells/cm² is recommended.

6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps 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.

Maintenance of Culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.
2. Change the medium every other day thereafter, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluence, change medium every day until the culture is approximately 90% confluent.

Subculture:

1. Subculture the cells when they are over 90% confluent.
2. Prepare cell culture flasks.
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°C waterbath prior to use.
4. Rinse the cells with DPBS.
5. Add 8 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).
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 to 2 minutes (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%.
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 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).