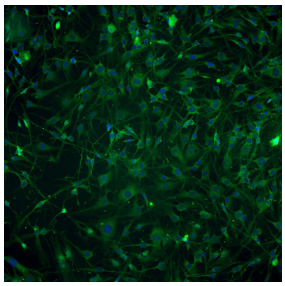
REF: P10869-IM

OCULAR CELL SYSTEM INNOPROFILE™
IMMORTALIZED HUMAN RETINAL PERICYTES



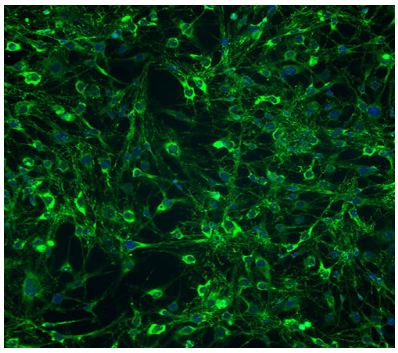
Product Type:	Immortalized Retinal Vascular Pericytes
Catalog Number:	P10869-IM
Source:	Human Retinal Tissue
Number of Cells:	1 x 10^6 Cells / vial (1ml)
Storage:	Liquid Nitrogen

P10869-IM have been obtained immortalizing Primary Human Retinal Pericytes with Lenti-SV40 Lentivirus. Immortalized cells were controlled passaging side by side with the primary cells. Primary cells go into senescence after the 5th passage while the SV40-tranduced 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 microvescular-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
  (Reference: P60121)



Product Characterization

Immunofluorescent method

- α-smooth muscle actin
- PDGF-b
- Vimentin

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

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edf. 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37 ºC 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 µg/cm2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 150 µl of poly-L-lysine stock solution (1 mg/ml, Innoprot cat. no. PLL). Leave the flask in incubator overnight (minimum two hours at 37ºC 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 37ºC 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 higher than 7,500 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 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 normal smooth muscle 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.

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Subculture:

1. Subculture the cells when they are over 90% confluent.

2. Prepare poly-L-lysine coated cell culture flasks (2 µg/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 37ºC waterbath prior to use.

Note: DPBS, trypsin/EDTA solution & trypsin neutralization solution are included in the "Primary Cells Detach Kit provided by Innoprot (Cat. Nº P60305).

4. Rinse the cells with DPBS.

5. Add 10 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37ºC incubator for 1 to 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS); transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the flask at 37oC for 1 minutes (no solution in the flask at this moment); at the end of trypsinization, one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.

6. Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (Beckman Coulter Allegra 6R centrifuge or similar) for 5 min; re-suspend cells in growth medium.

7. Count cells and plate cells in a new, poly-L-Lysine coated flask with cell density as recommended..

Caution: *Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, 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).

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