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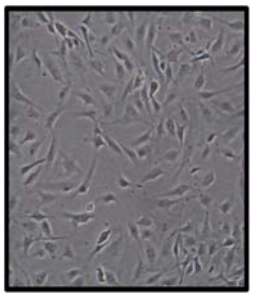
株式会社ケーエーシー
KAC

REF: P10861-IM

NEUROSCIENCE INNOPROFILE™

IMMORTALIZED HUMAN DERMAL MICROVASCULAR ENDOTHELIAL CELLS



Product Type:	Immortalized Dermal Endothelial Cells
Catalog Number:	P10861-IM
Immortalization:	SV40 Large T Antigen
Number of cells:	> 1 x10^6 cells in Cryopreserved vials
Storage:	Liquid Nitrogen

P10861-IM have been obtained immortalizing Human Dermal Microvascular Endothelial Cells with Lenti-SV40 Lentivirus. Immortalized cells were controlled passaging side by side with the primary cells. Primary cells go into senescence after the 3rd passage while the SV40-tranduced cells go beyond 30 passages.

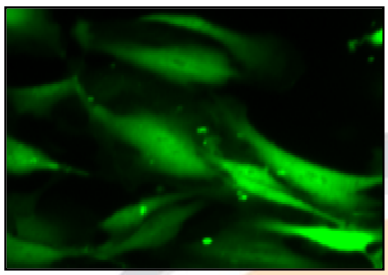
The predominant cell type in all skin blood vessels is the endothelial cell. Endothelial cells form the interface between intravascular and extravascular compartments and serve as a selective barrier for the diffusion of cells and macromolecules between these two compartments. The endothelial cells lining blood vessels of the skin are active participants in a variety of physiological processes, including wound healing, control of hemostasis, temperature regulation, and the modulation of inflammation/leukocyte trafficking. Endothelial cell proliferation, quiescence, apoptosis, and senescence are the four principal cytologic states that set the cutaneous microvascular in dynamic balance between maintenance and remodeling.

Product Characterization

Positive for:

- CD31 (P-CAM)
- CD36
- CD44
- ICAM-1
- vWF/Factor VIII

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi



Product Use

Sale of this item is subjected to the completion of a Material Transfer Agreement (MTA) by the purchasing individual/institution for each order. If you have any questions regarding this, please contact us at innoprot@innoprot.com

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

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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 fibronectin coated flask (2 µg/cm^2, T-75 flask is recommended). Add 10 ml of sterile Dulbecco's phosphate buffered saline (DPBS, Ca++ and Mg++ free) to a T-75 flask and then add 150 µl of fibronectin stock solution (1 mg/ml, Innoprot cat. no. P8248). Leave the flask in incubator overnight.

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. Aspirate fibronectin solution and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells. The fibronectin solution can be used twice.

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, fibronectin coated culture vessels. A seeding density of 10,000 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 DMSO residue in the culture. It is also important that endothelial cells are plated in fibronectin coated flask that promotes cell attachment and growth.

6. Replace the cap or cover, 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 cobblestone or spindle shaped morphology, non-granular 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.

2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.

3. Once the culture reaches 70% confluence, change medium every other day until the culture is approximately 90% confluent.

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Subculture:

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

2. Prepare fibronectin coated flasks (2 µg/cm^2) one day before subculture.

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.

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; 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 trypsinisation, 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%.

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

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, fibronectin 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).

株式会社ケー・エー・シー
試薬事業部
〒661-0978　兵庫県尼崎市久々知西町2丁目1-20

（お問合せ先）
TEL：06-6435-9747　FAX：06-6435-9748
URL：https://www.kacnet.co.jp/
E-mail：shiyaku-info@kacnet.co.jp