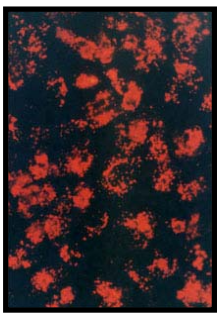
REF: P10338

NEUROSCIENCES INNOPROFILE™

BOVINE BRAIN MICROVASCULAR ENDOTHELIAL CELLS



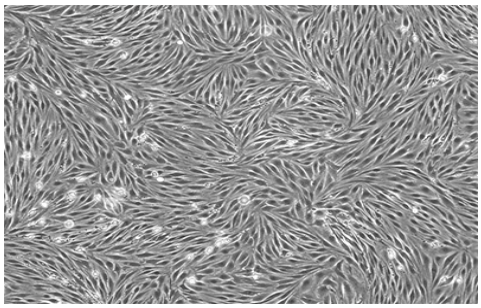
Product Type:	Cryo-preserved Brain Endothelial Cells
Catalog Number:	P10338
Source:	Bovine Brain
Number of cells:	> 5 x10^5 cells in Cryopreserved vials
Storage:	Liquid Nitrogen

Bovine Brain Microvascular Endothelial Cells (BBMEC) from Innoprot are isolated by ScienCell Research Laboratories from healthy bovine brain. BBMEC are cryopreserved at passage one and delivered frozen. BBMEC are guaranteed to further expand for 5 population doublings under the conditions provided in the technical sheet.

The Brain MEC are the major element of the blood-brain barrier and comprise the primary limitation to passage of substances, both soluble and cellular, from the blood into the brain. Brain MEC utilizes unique features that distinguish themselves from those of peripheral endothelial cells. Like peripheral endothelial cells, however, brain MEC express, or can be induced to express, cell adhesion molecules on their surface that regulate the extravasation of leukocytes into the brain. In view of the organ specificity of MEC, Brain MEC has been widely used for studying the molecular and cellular basis of blood-brain barrier.

Recommended Medium

- Endothelial Cell Medium(Cat. P60104)



Product Characterization

Immunofluorescent method

- vWF/Factor VIII
- CD31 (P-CAM)

By uptake of DiI-Ac-LDL

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.
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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!
NOTE: *BBMEC are very sensitive cells and they are not expected to proliferate many times in culture. Experiments should be well organized before thawing the cells. Do not allow cells to become 100% confluent before subculturing. We recommend subculturing cells at 80% confluency.*

Set up culture after receiving the order:

1. Prepare a fibronectin coated flask (2 µg/cm², 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, Ref: 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 15 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 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.

5. Remove the cap, being careful not to touch the interior threads with fingers. Using a 1 ml eppendorf pipette gently re-suspend the contents of the vial.

6. Dispense the contents of the vial into the equilibrated, fibronectin coated culture vessels. A minimum seeding density of 7,500 cells/cm² 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.

7. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.

8. Return the culture vessels to the incubator.

9. 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. 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 80% confluent.

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Subculture:

1. Subculture the cells when the culture reaches 80% confluency.

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 37°C waterbath prior to use.

4. Rinse the cells with DPBS.

5. Add 10 ml of DPBS and then 0.5 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;

7. 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 37°C for another 1 to 2 minutes (no solution in the flask at this moment).

8. At the end of incubation, 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.

9. 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.

10. 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%.

11. 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).

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