Bovine Brain Microvascular Endothelial Cells (BBMEC)

Catalog #B1000

Cell Specification

Brain microvascular endothelial cells (BMEC), the major component of the blood-brain barrier, limit the passage of substances, both soluble and cellular, from the blood into the brain. BMEC utilize unique features to distinguish themselves from peripheral endothelial cells, such as 1) intercellular tight junctions that display high electrical resistance and slow paracellular transport, 2) the absence of fenestrae and a reduced level of pinocytic activity, and 3) the expression of specialized pumps that can transport compounds out of the brain via the blood-brain barrier [1-3]. Similar to peripheral endothelial cells, BMEC express, or can be induced to express, cell adhesion molecules on their surface that regulate the extravasation of leukocytes into the brain. Cultured bovine BMEC have been used for studying the molecular and cellular properties of blood-brain barrier because of their unique functions [4]. Understanding the molecular mechanisms of blood-brain barrier regulation may help to optimize drug delivery to the CNS and elucidate new therapies for CNS diseases.

BBMEC from ScienCell Research Laboratories are isolated from bovine brain. BBMEC are cryopreserved at passage one and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. BBMEC are characterized by immunofluorescence with antibody specific to vWF/Factor VIII. BBMEC are negative for mycoplasma, bacteria, yeast, and fungi. BBMEC are guaranteed to further expand for 5 population doublings under the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use Endothelial Cell Medium (ECM, Cat. #1001) for culturing BBMEC *in vitro*.

Product Use

BBMEC 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] Crone C, Oleson SP. (1992) "Electrical resistance of brain microvessel endothelium." *Brain Res*. 241: 49-55.

[2] Reese TS, Karnovsky MJ. (1967) "Fine structural localization of blood-brain barrier to exogenous peroxidase." *J Cell Biol*. 34: 9-14.

[3] Wolburg H, Neuhaus J, Kniesel U, Kraub B, Schmid EM, Ocalan M, Farrell C, Risau W. (1994) "Modulation of tight junction structure in blood-brain barrier endothelial cells." *J Cell Sci*. 107: 1347-1357.

[4] Dorovini-Zis K, Bowman P, Betz A, Goldstein G. (1987) "Formation of a barrier by brain microvessel endothelial cells in culture." Fed Proc. 46(8): 2521-2522.

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!

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.

Initiating the culture:

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, Ca^{++} and Mg^{++}-free (ScienCell, Cat. #0303) to a T-75 flask and then add 150 μl of fibronectin stock solution (ScienCell, Cat. #8248). Leave the vessel in a 37 °C incubator overnight.
2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.
3. Aspirate fibronectin solution and add 15 ml of complete medium to the culture vessel. The fibronectin solution can be used twice. 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, fibronectin-coated culture vessel. A seeding density of 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 residual DMSO in the culture. It is also important that cells are plated in fibronectin-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.

2. Change the medium every two days thereafter, until the culture is approximately 80% confluent.

Subculturing:

1. Subculture when the culture reaches 80% confluency.
2. Prepare fibronectin-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 37ºC water bath 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 37ºC 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 37ºC 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%.

    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 fibronectin-coated culture vessel with the recommended cell density.

Caution: Handling animal derived products is potentially biohazardous. 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: 191-199.