Rev. 1

Rat Brain Microvascular Endothelial Cells (RBMEC)

Catalog #R1000

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 rat BMEC have been widely used for studying the molecular and cellular properties of blood-brain barrier because of their unique functions. 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.

RBMEC from ScienCell Research Laboratories are isolated from adult CD® IGS rat brain. RBMEC are cryopreserved at passage one and delivered frozen. Each vial contains >5 x 10^5 cells in 1 ml volume. RBMEC are characterized by immunofluorescence with antibodies specific to vWF and/or CD31 (PECAM). RBMEC are negative for mycoplasma, bacteria, yeast, and fungi. RBMEC 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-rat (ECM-r, Cat. #1021) for culturing RBMEC *in vitro*.

Product Use

RBMEC 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.

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Instructions for culturing primary cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Note: *RBMEC are very sensitive cells and they are not expected to proliferate many times in culture. Experiments should be well organized before thawing the cells.*

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37ºC, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

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 ºC 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 20 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 37ºC 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.

   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. It is 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.

Maintaining the culture:

1. Refresh supplemented culture 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% confluency, change medium every other day until the culture is approximately 80-90% confluent.

Subculturing:

1. Subculture when the culture reaches 90% confluency.
2. Prepare fibronectin-coated culture vessels (2 μg/cm^2) one day before subculture.
3. Warm complete medium, trypsin/EDTA solution, 0.05% (T/E, Cat. #0183), 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 37°C water bath prior to use.
4. Rinse the cells with DPBS.
5. Add 8 ml DPBS and 2 ml 0.05% T/E solution (Cat. #0183) into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Use a microscope to monitor the change in cell morphology.

   Note: We recommend using ScienCell's 0.05% T/E solution, which is optimized to minimize cell damage due to over trypsinization. If 0.25% T/E solution (Cat. #0103) is used, then 9.6 ml of DPBS and 0.4 ml of 0.25% T/E solution should be used.

   Caution: Do NOT use undiluted trypsin when subculturing primary cells.
6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum (FBS, Cat. #0500).
7. Once the cells completely round up, transfer T/E solution from the flask to a 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37°C for another minute (no solution in the flask at this time).
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. Gently resuspend cells in culture medium.
12. Count and plate cells in a new fibronectin-coated culture vessel with the recommended cell density. A seeding density of 10,000-12,000 cells/cm^2 is recommended.

Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

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: 191-9.