

**SOP:** Propagation of Human Brain Microvascular Endothelial Cells (HBMEC, ScienCell Research Laboratories)  
**Date modified:** 05/04/2010  
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### **Ordering Information**

Human Brain Microvascular Endothelial Cells (HBMEC) may be ordered as frozen ampoules. Each vial contains  $>5 \times 10^5$  cells in a 1ml volume.

To order frozen ampoules + media:

Name:	HBMEC – Human Brain Microvascular Endothelial Cells
Item #:	1000 (HBMEC - Cryopreserved ampoule) 1001 (ECM, Endothelial Cell Medium)

### **Notes:**

The number of media kits purchased depends on the target number of cells to be generated. A rule of thumb is 10 media kits for every initial cryopreserved ampoule. It is strongly recommended to purchase all of the media that will be required for a complete expansion series, since media supply may be erratic. All culture flasks must be coated with fibronectin ( $2\mu\text{g}/\text{cm}^2$ ).

### **Materials List**

1. ECM Endothelial Cell Medium (ScienCell, Cat# 1001)
2. Fibronectin (0.1% Solution from Bovine Plasma, Sigma-Aldrich, Cat# F1141)
3. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
4. Accutase Enzyme Cell Detachment Medium (EBioscience, Cat# 00-4555)
5. T75, T225 culture flasks
6. Graduated pipets (1, 5, 10, 25, 50mL)
7. Pen-Strep solution (if required; ScienCell typically supplies antibiotics)
8. Hemocytometer
9. Micropipet w/ P20 tips
10. Microscope

### **Procedure**

#### **A. Initiation of culture from cryopreserved cells**

- 1) Rapidly thaw cells by holding vial and gently rotating in  $37^\circ\text{C}$  water bath.
- 2) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then dispense contents of vial into a flask at  **$7,500 \text{ cells}/\text{cm}^2$  density**.
- 3) Let cells recover for 16 hours in a  $37^\circ\text{C}$ , 5%  $\text{CO}_2$  humidified incubator.
- 4) The next morning, the diluted DMSO-containing shipping/cryopreservation medium is aspirated from the cell layer and replaced with fresh medium.

#### **B. Sub-culture**

- 1) Propagate cells until density reaches 80% confluence.
- 2) Aspirate medium.
- 3) Wash cells with warm 1X PBS.
- 4) Add 15mL of Accutase and return to incubator for 10-15 minutes, or until cells detach.
- 5) Immediately remove cells, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4°C).
- 6) Gently re-suspend cell pellet in warm medium.
- 7) Count cells with hemocytometer.
- 8) Add warmed medium to flasks.
- 9) Seed flasks at **7,500 cells/cm<sup>2</sup> density**.
- 10) Record each subculture event as a passage.

### C. Maintenance

- 1) Change media the day after seeding and every OTHER day thereafter.
- 2) Increase media volume as confluency increases (volumes assume the use of
- 3) T225 flasks):
  - a. 25% = 1mL/5 cm<sup>2</sup>
  - b. 25-45% = 1.5mL/5 cm<sup>2</sup>
  - c. 45%+ = 2mL/5 cm<sup>2</sup>.
- 4) Per the above an exemplary schedule might be:
  - a. day 1, plate into T225: use 50mL of media.
  - b. day 2, change media, use 50mL of media
  - c. day 4, change media, use 100mL of media (if confluency is >50%)
  - d. day 6, change media, use 100mL of media (or harvest if ready)
  - e. day 7 or 8 (harvest when cells reach  $6 \times 10^6$  cells/flask).

### D. Harvest

- 1) Pass cells 3-4 times until the desired cell number is achieved (primary cells will senesce after 4-5 passages).
- 2) Remove cells from flasks according to protocol described above under 'Sub-culture'.
- 3) Examine viability using Trypan blue staining (SOP TP-7).