

**SOP:** **Propagation of Normal Adult Human Lymphatic Microvascular Endothelial Cells, Dermal-Derived (Lonza Bioscience)**  
**Date modified:** **03/01/2010**  
**Modified by:** **E. Giste (UW)**

### **Ordering Information**

Normal Adult Human Lymphatic Microvascular Endothelial Cells, Dermal-Derived (HMVEC-dLy Ad) may be ordered either as frozen ampoules or as starter cultures. The former contain  $>5 \times 10^5$  cells; the latter are initiated at Lonza and sent in a T75 flask containing  $\sim 2 \times 10^6$  cells.

To order frozen ampoules + media:

Name: HMVEC-dLy Ad – Normal Adult Human Lymphatic  
Microvascular Endothelial Cells, Dermal-Derived  
Item #: CC-2810 (HMVEC-dLy Ad - Cryopreserved ampoule)  
CC-3202 (EGM-2 MV™ BulletKit® = CC-3156 + CC-4147)

To order starter cultures:

Name: HMVEC-dLy Ad – Normal Adult Human Lymphatic  
Microvascular Endothelial Cells, Dermal-Derived  
Item #: CC-2810T75 (HMVEC-dLy Ad in EGM-2 MV™ T75 Flask)  
CC-3202 (EGM-2 MV™ BulletKit® = CC-3156 + CC-4147)

### **Notes:**

The number of BulletKits purchased depends on the target number of cells to be generated. A rule of thumb is 10 BulletKits for every initial T225 flask of cells. 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.

### **Materials List**

1. Cell-type specific medium (BulletKits – Lonza Biosciences)
2. T75, T225 culture flasks
3. Graduated pipets (1, 5, 10, 25, 50mL)
4. Pen-strep solution (if required; Lonza typically supplies antibiotics)
5. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
6. Accutase Enzyme Cell Detachment Medium (EBiosciences Cat# 00-4555)
7. Hemocytometer
8. Micropipet w/ P20 tips
9. Microscope

### **Procedure**

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

- 1) Rapidly thaw cells by holding vial and gently rotating in 37°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 5,000 cells/cm<sup>2</sup> density.
- 3) Let cells recover for 16 hours in a 37°C, 5% CO<sub>2</sub> 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 60-80% 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 **5,000 cells/cm<sup>2</sup>**.
- 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 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>.
- 3) Per the above an exemplary schedule might be:
  - a. day 1, plate into T225: use 50 mls of media.
  - b. day 2, change media, use 50 mls of media
  - c. day 4, change media, use 100 mls of media (if confluency is >50%)
  - d. day 6, change media, use 100 mls of media (or harvest if ready)
  - e. day 7 or 8 (harvest when cells reach 6 x 10<sup>6</sup> 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).