**SOP:** **Propagation of Normal Adult Human Microvascular Endothelial Cells, Dermal-Derived (HMVEC-d Ad, Lonza Biosciences)**
**Date modified:** **05/23/2011**
**Modified by:** **T. Canfield (UW)**

## Ordering Information

Normal Adult Human Microvascular Endothelial Cells, Dermal-Derived (HMVEC-d Ad) may be ordered either as frozen ampoules or as starter cultures. The former contain >5 x $10^5$ cells; the latter are initiated at Lonza and sent in a T225 flask containing 5-7 x $10^6$ cells.

To order frozen ampoules + media:
Name: HMVEC-d Ad – Normal Adult Human Microvascular Endothelial Cells, Dermal-Derived
Item #: CC-2543 (HMVEC-d Ad - Cryopreserved ampoule)
CC-3202 (EGM-2MV™ BulletKit® = CC-3156 + CC-4147)
To order starter cultures:
Name: HMVEC-d Ad – Normal Adult Human Microvascular Endothelial Cells, Dermal-Derived
Item #: CC2543T225 (HMVEC-d Ad in BGM-2MV™ T225 Flask)
CC-3202 (EGM-2MV™ 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. 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. Receipt of proliferating cells

1) Swab down flask with 70% ethanol.
2) Equilibrate for 3-4 hours in 37$^o$C, 5% $CO_2$ humidified incubator.
3) Remove shipping medium. Replace with fresh medium and return to incubator.

## B. Sub-culture

1) Propagate cells until density reaches 70-80% 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 ($4^oC$).
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/$cm^2$ 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 $cm^2$
    b. 25-45% = 1.5mL/5 $cm^2$
    c. 45%+ = 2mL/5 $cm^2$.
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 x $10^6$ 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).