

SOP: **Propagation of Normal Neonatal Human Microvascular Endothelial Cells (Single Donor), Dermal-Derived (Lonza Bioscience)**
Date modified: **02/01/2010**
Modified by: **E. Giste (UW)**

Ordering Information

Normal Neonatal Human Microvascular Endothelial Cells (Single Donor), Dermal-Derived (HMVEC-dNeo) 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 T225 flask containing $\sim 6-7 \times 10^6$ cells.

To order frozen ampoules + media:

Name: HMVEC-dNeo – Normal Neonatal Human Microvascular Endothelial Cells (Single Donor), Dermal-Derived
Item #: CC-2505 (HMVEC-dNeo - Cryopreserved ampoule)
CC-3202 (EGM-2-MV™ BulletKit® = CC-3156 + CC-4147)

To order starter cultures:

Name: HMVEC-dNeo – Normal Neonatal Human Microvascular Endothelial Cells (Single Donor), Dermal-Derived
Item #: CC-2505T225 (HMVEC-dNeo in EGM-2-MV™ T225 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. 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°C, 5% CO₂ humidified incubator.

- 3) Remove shipping medium. Replace with fresh medium and return to incubator.

B. Sub-culture

- 1) Propagate cells until density reaches 60-80% confluence.
- 2) Aspirate medium.
- 3) Wash cells with warm 1X PBS.
- 4) Add 15mLs of Accutase and return to incubator for 10-15 minutes.
- 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/cm²**
- 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 cm²
 - b. 25-45% = 1.5mL/ 5 cm²
 - c. 45%+ = 2mL/ 5 cm².
- 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 6×10^6 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).