SOP: **Propagation of primary Human Umbilical Vein Endothelial Cells (HUVECs; Lonza Biosciences)**
Date modified: **8/27/08**
Modified by: **M. Dorschner (UW)**
L. Dillon (NHGRI)

Ordering Information

Primary Human Umbilical Vein Endothelial Cells (HUVECs) may be ordered either as frozen ampules or as starter cultures. The former contain ~0.5-1 x 10^5 cells; the latter are initiated at Lonza and sent in a T225 flask containing 6-7 x 10^6 cells.

For all orders, provide (1) Reservation #; (2) Contract/quotation #; (3) Individual (Lot #); and (4) Item #s, as follows:

Reservation number: ~~RZ 495718~~ 3122124 (Updated 7/22/08)
Contract number: P101416
Individual H1: Lot #7F3239 - Male, Caucasian,
~~154 amps available~~, 0 amps available (7/22/08)

Individual H2: Lot #7F3771 - Male, African American
~~144 amps available~~, 38 amps available (7/22/08)

To order frozen ampules + media:
Name: HUVEC – Umbilical Vein Endo Cells
Item #: CC-2517 (HUVEC in EGM® - Cryopreserved ampule)
CC-3162 (EGM-2 BulletKit = CC-3156 + CC-4176)

To order starter cultures:
Name: HUVEC – Umbilical Vein Endo Cells
Item #: CC2501T225 (HUVECs in EGM® T-225 Flask)
CC-3162 (EGM-2 BulletKit = CC-3156 + CC-4176)

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 (see below), 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, 25mL)
4. Pen-strep solution (if required; Lonza typically supplies antibiotics)
5. Hemocytometer
6. Micropipet w/ P20 tips
7. Microscope

Procedure

A. Receipt of proliferating cells

1) Equilibrate for 3-4 hours in 37^{o}C, 5% CO_2 humidified incubator.
2) Remove shipping medium. Replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 3 minutes (4^{o}C)
6) Wash cells 2X with 1X PBS.
7) Gently re-suspend cell pellet in warm medium.
8) Count cells with hemocytometer.
9) Add warmed medium to flasks.
10) Seed flasks at **5,000 cells/cm^2**
11) Record each subculture event as a passage

C. Maintenance

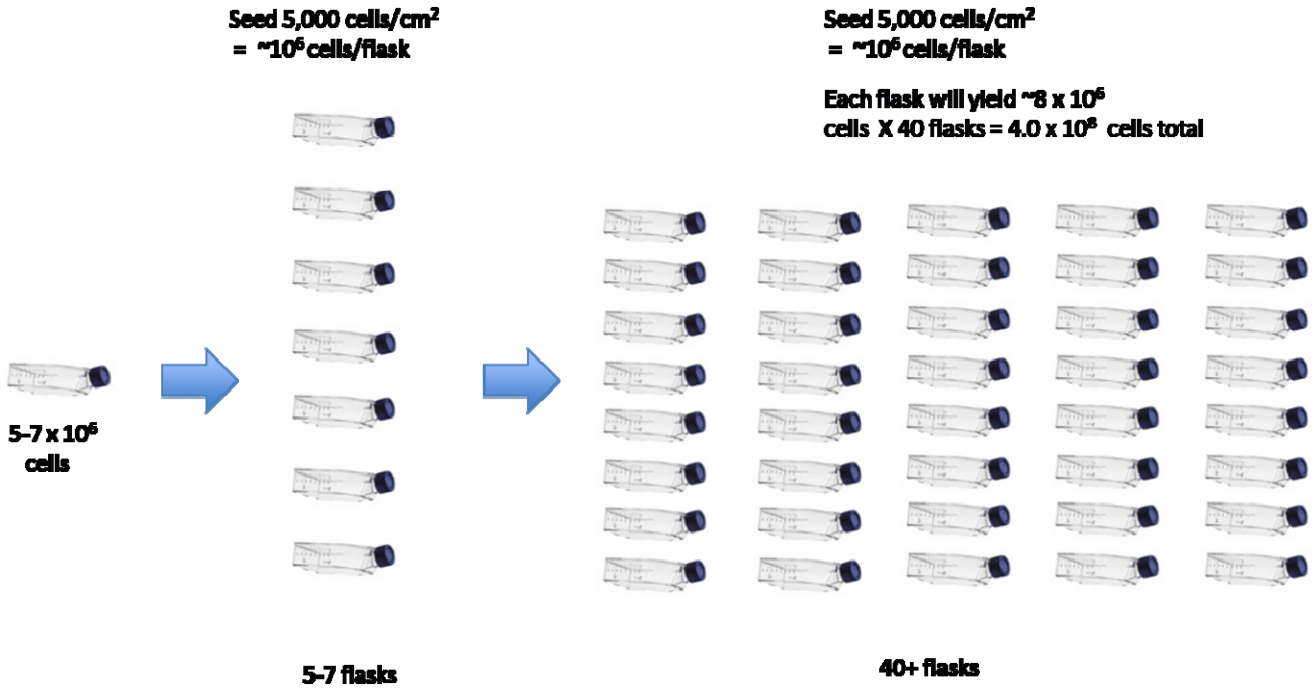
1) Change media the day after seeding and every OTHER day thereafter.
2) Increase media volume as confluency increases (volumes assume the use of
3) T225 flasks):
    a. 25 % = 1mL/5 cm^2
    b. 25-45% = 1.5mL/ 5 cm^2
    c. 45%+ = 2mL/ 5 cm^2
4) Per the above an exemplary schedule might be:
    a. day 1, plate into T225: use 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 10^6 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 'subculturing'
3) Examine viability using trypan blue staining (SOP)

Exemplary Expansion

The diagram below illustrates an exemplary expansion of HUVECs from a Lonza starter culture:



- The initial T225 flask received from Lonza will have ~6 x 10^6 cells; this will then be split and seeded at ~3,500 cells/cm^2; each new T225 flask will therefore be seeded with ~750K cells.
- The initial flask will yield up to 7-8 daughter flasks depending on how large of an expansion is targeted.
- Once these flasks have reached the target density again, they can be split and seeded into up to 40 flasks.
- The 40 granddaughter flasks will each yield ~6 x 10^6 cells, providing a total theoretical yield of 2.5x10^8 cells.

Media requirements: Each flask will require ~50mL of medium with additional medium for feedings during the doubling/expansion process.