

SOP: Propagation of primary Keratinocytes
(NHEK; Lonza Biosciences)
Date modified: 7/22/08
Modified by: M. Dorschner (UW)
L. Dillon (NHGRI)

Ordering Information

Normal Human Epidermal Keratinocytes (NHEKs) may be ordered either as frozen ampules or as starter cultures. The former contain $\sim 0.5-1 \times 10^5$ cells; the latter are initiated at Lonza and sent in a T225 flask containing $6-7 \times 10^6$ 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 K1: Lot #4F1155J - Female, African American
~~178 amps available~~, 51 amps available (7/22/08)

Individual K2: Lot #7F4307 - Female, Caucasian
~~179 amps available~~; 139 amps available (7/22/08)

To order frozen ampules + media:

Name: NHEK – Adult Keratinocytes
Item #: CC-2501 (NHEK in KGM® - Cryopreserved ampule)

CC-3111 (KGM BulletKit = CC-3101 + CC-4131)

To order starter cultures:

Name: NHEK – Adult Keratinocytes
Item #: CC2501T225 (NHEKs in KGM® T225 Flask)
CC-3111 (KGM BulletKit = CC-3101 + CC-4131)

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°C, 5% CO₂ 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°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 **3,500 cells/cm²**
- 11) Record each subculture event as a passage

C. Maintenance

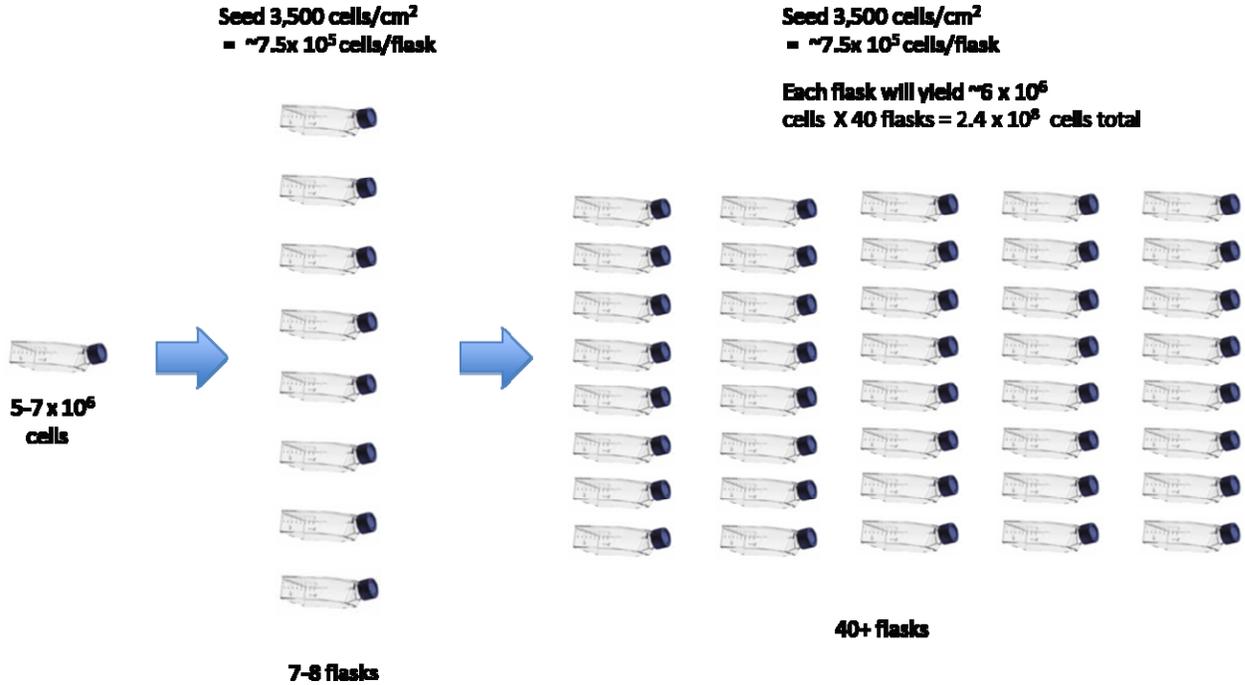
- 1) Change media the day after seeding and every OTHER day thereafter.
- 2) Increase media volume as confluency increases (volumes assume the use of
- 3) T225 flasks):
 - a. 25 % = 1mL/5 cm²
 - b. 25-45% = 1.5mL/ 5 cm²
 - c. 45%+ = 2mL/ 5 cm².
- 4) Per the above an exemplary schedule might be:
 - a. 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 10⁶ 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 TP-7)

Exemplary Expansion

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



- The initial T225 flask received from Lonza will have ~6 x 10⁶ cells; this will then be split and seeded at ~3,500 cells/cm²; 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 10⁶ cells, providing a total theoretical yield of 2.5x10⁸ cells.

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