SOP: **Propagation of Human Non-pigment Ciliary Epithelial Cells (ScienCell Research Laboratories)**
Date modified: **11/12/09**
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Ordering Information

Human Non-pigment Ciliary Epithelial Cells (HNPCEpiC) may be ordered as frozen ampoules. Each vial contains >5 x 10^5 cells in a 1ml volume.

To order frozen ampoules + media:

Name:	HNPCEpiC – Human Non-pigment Ciliary Epithelial Cells
Item #:	6580 (HNPCEpiC - Cryopreserved ampoule)
	4101 (EpiCM, Epithelial Cell Medium)

Notes:

The number of media kits purchased depends on the target number of cells to be generated. A rule of thumb is 10 media kits for every initial cryopreserved ampoule. 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. All culture flasks must be coated with poly-L-lysine (2 ug/cm^2).

Materials List

1. Cell-type specific medium (ScienCell)
2. Poly-L-Lysine (#0413, 10mg/ml, ScienCell)
3. T225 culture flasks
4. Graduated pipets (1, 5, 25, 50mL)
5. Pen-Strep solution (if required; ScienCell typically supplies antibiotics)
6. Accutase – Enzyme Cell Detachment Medium (cat # 00-4555, EBioscience)
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, dispense contents of vial into a flask at 5,000 cells/cm^2 density.
3) Let cells recover for 16 hours in a 37°C, 5% CO_2 humidified incubator.

B. Sub-culture

1) Propagate cells until density reaches 90% 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^{o}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^2. 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 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).