SOP: **Propagation of Normal Human Periodontal Ligament Fibroblast Cells (Lonza Bioscience)**
Date modified: **03/29/2010**
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Ordering Information

Normal Human Periodontal Ligament Fibroblast Cells (HPdLF) may be ordered as frozen ampoules. The frozen ampoules contain >5 x 10^5 cells.

To order frozen ampoules + media:

Name:	HPdLF– Normal Human Periodontal Ligament Fibroblast Cells
Item #:	CC-7049 (HPdLF - Cryopreserved ampoule)
	CC-3205 (SCGM™ BulletKit® = CC-3204 + CC-4181)

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 Bioscience)
2. T75, 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. Initiation of culture from cryopreserved cells

1) Rapidly thaw cells by holding vial and gently rotating in a 37°C water bath.
2) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then dispense contents of the vial into a flask at **3,500 cells/cm^2 density**.
3) Let cells recover for 16 hours in a 37°C, 5% CO_2 humidified incubator.
4) The next morning, the diluted DMSO-containing shipping/cryopreservation medium is aspirated from the cell layer and replaced with fresh medium.

B. Sub-culture

1) Propagate cells until density reaches 60-80% 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^{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 **3,500 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 T225 flasks):
    a. 25% = 1mL/5 cm^2
    b. 25-45% = 1.5mL/5 cm^2
    c. 45%+ = 2mL/5 cm^2.
3) 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).