

SOP: Propagation of Human Gingival Fibroblasts (HGF, ScienCell)
Date modified: 9/11/09
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Ordering Information

Human gingival fibroblasts (HGF) may be ordered as frozen ampoules. Each vial contains $>5 \times 10^5$ cells in a 1ml volume.

To order frozen ampoules + media:

Name:	HGF – Human gingival fibroblasts
Item #:	2620 (HGF - Cryopreserved ampoule)
	2301 (FM, Fibroblast 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 ($2\mu\text{g}/\text{cm}^2$).

Materials List

1. Cell-type specific medium (ScienCell)
2. Poly-L-lysine coated T225 culture flasks (T225 flasks treated with Poly-L-lysine, cat# 0413, 10mg/ml, ScienCell)
3. Accutase (eBioscience, cat# 00-4555-56)
4. Graduated pipets (1, 5, 25mL)
5. Pen-strep solution (if required)
6. Hemocytometer
7. Micropipet w/ P20 tips
8. 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 $5,000 \text{ cells}/\text{cm}^2$ 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) Decant medium.
- 3) Wash cells with warm 1X PBS.

- 4) Add 10-15mLs of Accutase and return to incubator for 10-15 minutes (or until cells have been released from flask; maximum incubation not to exceed 45 minutes).
- 5) Immediately remove cells and pellet at 500 xg for 5 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 **5,000 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)