

HFF-hTERT clone #6 (HFFc6) growth conditions

Growth Media (500 ml)

- 400 ml Dulbecco's Modified Eagle's Medium (DMEM)
- 100 ml Heat-inactivated FBS (20%)
- No antibiotics

Culture Conditions

Atmosphere (37°C):

- air, 95%;
- carbon dioxide (CO₂), 5%

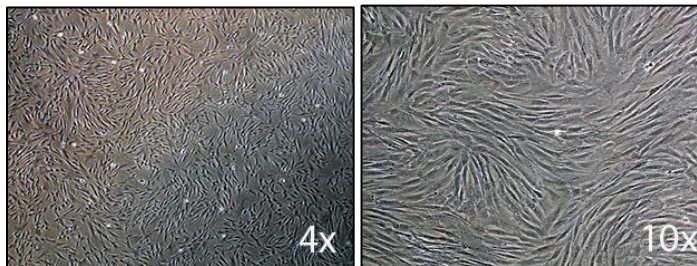


Figure 1: ~90% confluency

Figure 2: Overgrown

Thawing initial stock

Initial stock vial contains 0.5-1.0 x10⁶ viable cells. Thaw the vial quickly in a 37°C water bath, transfer the contents to a 15 ml conical tube containing 5 ml of warm media, spin (250xg for 5 min at room temp.), remove the supernatant and resuspend the pellet in 5 ml media. Plate all the cells into one T25 flask or equivalent area (at 10⁴ cells/cm² minimum).

Sub-culturing

HFFc6 cells¹ are split when they are ~90% (Figure 1) confluent (~every 2-3 days at a 1:4 dilution)

Please take pictures after thawing and from the first 90% confluent sub-culture. Cells should not overgrow the plate.

Overgrown cultures will have elongated cells growing in “waves” (Figure 2).

Procedure

Before doing the subculture, media and Accutase® are placed at room temperature or at 37°C before using it on the cells

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 1X DPBS
3. Add 1.5mL mL of Accutase® solution to T75 flask and incubate for 2-3 minutes.
4. Add 8.5 mL of complete growth medium and rinse surface of the flask to detach all cells. Gently pipetting up and down to re-suspend the cells.
5. Add 2.5 mL of the cell suspension to a new T75 and complete with media.
6. Place flasks in incubator at 37°C with a 5% CO₂ in air atmosphere.

Flask/plate	Number of cells seeded	Number of cells at confluency
T25	2.5 x10 ⁵	1.0 x10 ⁶
T75	7.5 x10 ⁵	3.0 x10 ⁶
10 cm Ø	7.8 x10 ⁵	3.1 x10 ⁶
15 cm Ø	1.8 x10 ⁶	7.5 x10 ⁶

Freezing/thawing

- Cells can be frozen using Bambanker solution (see manufacturer's instructions)

Ordering info

- DMEM: high glucose, GlutaMAX™ Supplement, pyruvate, (Fisher Scientific: #10569010
- Heat-inactivated FBS (Premium grade US origin, 500 ML, HI [Seradigm AB through **VWR**, [item: 97068-091, Quote:8030223666, reserve: 8003216852, Lot: 035B15](#)])
- Accutase (StemPro® Accutase® Cell Dissociation Reagent, Fisher Scientific : #A1110501)
- DPBS (Fisher Scientific: #14190144)
- Bambanker cell freezing solution (Fisher Scientific/ Wako Chemicals USA, Inc.: 30214681)

¹ HFFc6 is a subclone of HFF-hTERT [MOLECULAR AND CELLULAR BIOLOGY, Apr. 2004, p. 2842–2852]