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:

- air, 95%;
- carbon dioxide (CO_2), 5%

Temperature: 37°C

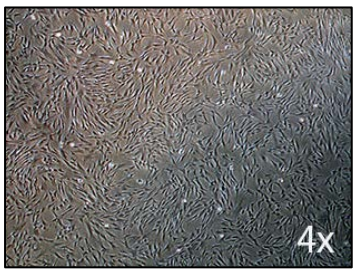

Figure 1: ~90% confluency

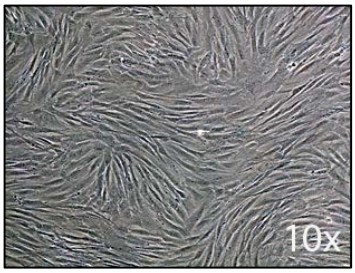

Figure 2: Overgrown

Sub-culturing

HFFc6 cells[1] 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% CO_2 in air atmosphere.

Flask/plate	*Number of cells seeded*	*Number of cells at confluency*
T75	$0.75.10^6$	3.10^6
15 cm diameter	$1.8.10^6$	$7.5.10^6$

Freezing

- Cell can be frozen DMSO-free using Bambanker solution (see manufacturer's instructions)

Ordering info

- DMEM: high glucose, GlutaMAX™ Supplement, pyruvate, (Fisher Scientific: #10569010
- Heat-inactivated FBS (FBS, Premium grade US origin, 500 ML, HI [Seradigm AB, **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]