

Code:	0275
Cell Line:	HFF-1
Species:	Homo sapiens Vulgar Name: Human
Tissue:	Skin, Foreskin
Morphology:	Fibroblast
Disease:	Normal
Growth Properties:	Adherent
Sex:	Male
Age Ethnicity:	NEWBORN
Applications:	Can be used to produce feeder cells
Biosafey:	1
Additional info:	it is not recommended to use them past passage no. 50 (P50). It is recommended that the feeder cells be plated 24 hours before use at 5X10 ⁴ cells/cm ² in order to obtain a supportive monolayer for stem cell growth.
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 4 mM L-glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate and 15% FETAL BOVINE SERUM.

Subculturing:

Cells should be split when they reach confluency. A split ratio of 1:5 to 1:7 is recommended. Volumes used in this protocol are for 150 or 225 cm²; proportionally reduce or increase amount of dissociation medium for culture flasks of other sizes.

Remove and discard culture medium.

Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum, which contain trypsin inhibitor.

Add 5 mL of Trypsin-EDTA (0.25% (w/v) Trypsin-0.53 mM EDTA solution to flask and incubate for 1 minute, gently tapping the flask observe cells under an inverted microscope until cells detach (usually within 1 to 2 minutes).

Add 6.0 to 8.0 mL of complete growth medium and rinse surface of the flask to detach all cells. Gently pipetting up and down will break cell clumps.

Transfer all cells into a centrifuge bottle or tube and centrifuge at 270 xg for 5 minutes.

Remove and discard the supernatant.

Add 10 mL complete growth medium to cell pellet and with 10 mL pipette resuspend the cells gently (create a single-cell suspension).

Add more complete growth medium to cell suspension as needed to plate cells at approximately 5x10⁶/T225 flask.

Place flasks in incubator 37°C with a 5% CO₂ in air atmosphere.

NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.

Medium Renewal: Twice a week or as pH decreases

Subcultivation ratio: 1:5 to 1:7 is recommended.

Culture Conditions: Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C

Cryopreservation: 95% FBS + 5% DMSO (Dimethyl sulfoxide)

**Thawing Frozen
Cells:**

SAFETY PRECAUTION: It is highly recommended that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that

some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. For cells that are sensitive to DMSO it is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio).
5. Incubate the culture in an appropriate atmosphere and temperature (see "Culture Conditions" for this cell line).

NOTE: It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

References:

Amit M, et al. Human feeder layers for human embryonic stem cells. *Biol. Reprod.* 68: 2150-2156, 2003; Hovatta O, et al. A culture system using human foreskin fibroblasts as feeder cells allows production of human embryonic stem cells. *Hum. Reprod.* 18: 1404-1408, 2003; Andrews P, et al. Human embryonic fibroblast feeder cells. International Patent Application WO 03/078611 A1.

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