

Normal Human Lung Fibroblasts

A Cells, Media and Reagents Information

Lonza Cat No	Name	Containing
CC-2512	NHLF in FGM-2 Cryopreserved Cells	> 500,000 cells / Amp
CC-3132	FGM-2 BulletKit,	Fibroblast Basal Medium, 500 ml FGM-2 SingleQuots
CC-3131	Fibroblast Basal Medium	500 ml
CC-4126	FGM-2 SingleQuots	hFGF-B, 0.5 ml Insulin, 0.5 ml FBS, 10 ml GA-1000, 0.5 ml
CC-5034	ReagentPack	Trypsin/EDTA, 100 ml HEPES Buffered Saline Solution, 100 ml Trypsing Neutralizing Solution, 100 ml

B Preparation of Media

- 1 Decontaminate the external surfaces of all supplement vials and the medium bottle with 70% ethanol .
- 2 Aseptically open each supplement vial and add the entire amount to the basal medium with a pipette.
- 3 Rinse each cryovial with the medium.
- 4 Record the expiration date (one month from the preparation data) on the medium bottle.

C Thawing of Cells/Initiation of Culture Process

- 1 Recommended seeding density for NHLF is 2500 cells / cm²
 - 2 Determine the total number of flasks by following equation.
Total # of flasks = Total Cell Count x Percent Viability x Seeding Efficiency / Growth Area x Rec. Seeding Density
 - 3 Add 15 ml medium to T75 (1 ml / 5 cm²) to equilibrate at 37C, CO₂, 5% for 30 min.
 - 4 Quickly thaw the cryovial in a 37C water bath. (Do not submerge it and remove it as soon as the ice melts)
 - 5 Resuspend cells in cryovial using a micropipette and transfer to the T75 set up earlier,.
 - 6 Gently rock T75 then place it back into incubator.
- Note Centrifugation should not be performed because centrifugation is more damaging than residual DMSO in the culture.

D Subculturing and Maintenance

- 1 Subculture when cells are ~ 80% confluent and contain many mitotic figures throughout the flask after 4 or 5 days growth.
- 2 Aliquot stated volume medium and reagents as listed below and warm to room temperature.

Reagents	T75 Flask	T175 Flask	150 mm Dish
Trypsin/EDTA	2 ml	4 ml	3 ml
HEPES buffered Saline Solution	15 ml	20 ml	15 ml
Trypsing Neutralizing Solution	4 ml	8 ml	6 ml
Growth Medium	15 ml	35 ml	25 ml

The following instructions are for a T 75 flask. Adjust all volumes accordingly for other size culture vessels.

- 3 Aspirate medium from the culture vessel.
- 4 Rinse the cells with 10 ml of room temperature HEPES-BSS.
- 5 Aspirate the HEPES-BSS from the flask.
- 6 Cover cells with 2 ml of Trypsin/EDTA solution.
- 7 Keep T75 in incubator for 2 to 6 minutes.
- 8 Examine the cell layer microscopically.
- 9 When ~ 90% cells rounded up, rap the flask against palm of hand to release the majority of cells from the culture surface.
- 10 After cells are released, neutralize the trypsin with 4 ml trypsin neutralizing solution.
- 11 Re-suspend cells up and down several times with 10 ml pipet.
- 12 Quickly transfer cells to centrifuge tube (15 ml or 50 ml).
- 13 Rinse flask with 5 ml of HEPES-BSS, combine all cells.
- 14 Microscope examine the harvested flask to make sure the cells left behind be less than 5%.
- 15 Centrifuge at 220 xg for 5 min at RT to pellet the cells.
- 16 Aspirate most supernatant, except of 100 - 200 ul and flick the tube with finger to loosen pellet.

- 17 Resuspend cells with 5 ml to 10 ml medium and mix with 5 ml or 10 ml pipet to ensure a uniform suspension.
- 18 Determine cell number and viability(if necessary more dilute cells with HEPES-BSS to count)
- 19 Determine the total number of flasks to inoculate by using the following equation.
Total # of flasks to inoculate = Total # of viable cells / Growth area x Rec. seeding Density
- 20 Transfer the appropriate amount of growth medium (1 ml / 5 cm²) to the new vessels and warm in incubator for 30 min.
- 21 Resuspend cells with 5 ml or 10 ml pipet about 10 times to make sure cells separated each other very well.
- 22 Dispense the calculated volume into the prepared subculture flasks.
- 23 Place the new culture vessels back into a 37C humidified incubator with 5% CO₂ .
- 24 Change medium the day after seeding, and every other day.
Increase media volume as confluency increase as listed below .

Cells Confluence	Medium Volume / Area	T75 Flask	T175 Flask	150 mm Dish
< 25%	1 ml/ 5cm ²	15 ml	35 ml	25 ml
25 ~ 45%	1.5 ml/ 5cm ²	25 ml	50 ml	40 ml
> 45%	2 ml/ 5cm ²	30 ml	70 ml	50 ml

E Large Scale Harvest (> 2E+8 cells)

- 1 Thaw 1 Cryovial NHLF cells [> 5 E+5cells / Amp] and plate into two T75 flasks .
- 2 Change fresh medium next day.
- 3 Check cell confluence every day. When cells are ~ 80% confluent (need 4 to 6 days growth), subculture cells (as described above under subculturing) into new vessels.
Each T75 flask can yield ~ 2E+6 cells.
- 4 Count cells with hemocytometer and seed as recommended seeding density (2500 cells / cm²) into need number T175 flasks
Total number of flasks depends upon cell yield and seeding density.
Each T175 flask can yield ~ 5E+6 cells.
- 5 Subculture cells 1 or 2 more times until the desired cell number (> 5 E+7 cells) is achieved for final harvesting (> 2 E+8 cells).
- 6 Subculture when these flasks have reached 80% confluence. Each T175 flask can yield ~ 5E+6 cells.
Seed cells as recommended seeding density (2500 cells / cm²) into needed # of 150 mm dishes.
(can be up to 50 x 150 mm dishes)
- 7 When ~ 80% confluent (need 4 days) harvest all cells (> 2 E+8 cells).
Each 150 mm dish can yield ~ 3.6 E+6 cells.