Normal Human Dermal Fibroblasts, Adult

A Cells, Media and Reagents Information

Lonza Cat No	Name	Contain
CC-2511	NHDF -Ad Cryopreserved Cells	> 500,000 cells / Amp
CC-3132	FGM-2 BulletKit,	Fibroblast Cell Basal Medium,500 ml FGM-2 SingleQuots,
CC-3131	Fibroblast Cell 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 Trypsin Neutralizing Solution (TNS), 100 ml

B Preparation of Media

1 Decontaminate the external sufraces 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 date) on the medium bottle.

C Thawing of Cells / Initiation of Culture Process

1 Recommended seeding density for NHDF is 3500 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, Do not keep longer after ice melt)

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 Aliquote stated volume medium and reagents as listed below and warm to room temperature.

Cell Culture Vessels	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
Trypsin Neutralizing Solution	4 ml	8 ml	6 ml
Growth Medium	15 ml	35 ml	25 ml

The following instructions are for a T75 falsk. 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 pippet.

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 are 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 innoculate = 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 Resupend cells with 5 ml or 10 ml pipet about 10 time to make sure cells seperated each other very will.

22 Dispense the calculated volume into the prepared subculture falsks.

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 bellow .

Cells Confluence	Medium Volume / Area	T75 Flask	T175 Flask	150 mm Dish
< 25%	1 ml/ 5cm2	15 ml	35 ml	25 ml
25 ~ 45%	1.5 ml/ 5cm2	25 ml	50 ml	40 ml
> 45%	2 ml/ 5cm2	30 ml	70 ml	50 ml

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

1 Thaw 1 Cryovial NHDF 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 ~ 1.2E+6 cells.

4 Count cells with hemocytometer and seed as recommended seeding density (3500 cells / cm2) into need number T175 flasks
Total number of flasks depends upon cell yield and seeding density.
Each T175 flask can yield ~3. 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 ~ 3.5E+6 cells.
Seed cells as recommended seeding density (3500 cells / cm2) into needed # of 150 mm dishes.
(can be up to 130 x 150 mm dishes)

7 When ~ 80% confluent (need 4 days) harvest all cells (> 2 E+8 cells).
Each 150 mm dish can yeild ~ 2.4 E+6 cells.

E Cell Image

NHDF cells (10x)