

Normal Human Skeletal Muscle Myoblasts

A Cells and Media Information

Lonza Cat No	Name	Contain
CC-2580	HSMM Cryopreserved	> 500,000 cells / Amp
CC-3245	SkGM-2 Bullet Kit	SkBM-2 Basal Medium SkGM-2 SingleQuots
CC-3246	SkBM-2 Basal Medium	500 ml
CC-3244	SkGM-2 SingleQuots	rhEGF, 0.5 ml Dexamethasone, 0.5 ml L-Glutamine, 10 ml FBS, 50 ml GA-1000, 0.5 ml
CC-5034	ReagentPack	Trypsin/EDTA Solution, 100 ml HEPES buffered Saline Solution, 100 ml Trypsin Neutralizing Solution (TNS), 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 HSMM 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 Recommended 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 flasks 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 50% ~ 70% confluent and contain many mitotic figures throughout the flask after 4 to 6 days growth.
- 2 Aliquot some volume medium and reagents as listed below then warm to room temperature.

Cell Growth vessels	T 75	T175	150 mm
Trypsin/EDTA	2 ml	4 ml	3 ml
D-PBS	15 ml	20 ml	15 ml
Trypsin Neutralizing Solution	4 ml	8 ml	6 ml
Aliquoted medium	15 ml	35 ml	25 ml

The following instructions are for a 75 cm² flask. Adjust all volumes accordingly for other size flasks.

- 3 Aspirate medium from the culture vessel.
- 4 Rinse the cells with 10 ml of room temperature D-PBS
- 5 Aspirate the D-PBS from the flask.
- 6 Cover cells with 2 ml of Trypsin/EDTA solution
- 7 Keep the T75 flask 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 5ml of D-PBS, 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 D-PBS 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 are separated from 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 bellow .

Cell confluence	medium volume / area	T75	T175	150 mm
< 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 (> 2 x 10⁸ cells)

- 1 Thaw one Cryovial HSMM cells [> 5 E+05 cells / Amp] into one T75 flask.
- 2 Change fresh medium next day.
- 3 Check cell confluence every day. When cells are ~ 70% confluent (need 4 to 6 days growth), subculture cells (as described above under subculturing) into new vessels.
Each T75 flask can yield ~ 2.9 E+06 cells.
- 4 Count total 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.
- 5 Subculture cells 1 or 2 more times until the desired cell number (> 5 E+07 cells) is achieved for final harvesting (> 2 E+08 cells).
- 6 Subculture when these flasks have reached 70% confluence. Each T175 flask can yield ~ 7.5 E+06 cells.
Seed cells as recommended seeding density (3500 cells / cm²) into needed # of 150 mm dishes.
- 7 When ~ 70% confluent (need 4 to 6 days) harvest all cells (> 2 E+08 cells).
Each 150 mm dish can yield ~ 2.6 E+06 cells.

F Cell Images

Cells before subculture [10 x]



Cells before subculture [10 x]

