SOP: **Propagation of Normal Human Skeletal Muscle Myoblasts (Lonza Bioscience)**
Date modified: **11/25/2010**
Modified by: **T. Canfield (UW)**

Ordering Information

Normal Human Skeletal Muscle Myoblasts (HSMM) may be ordered as frozen ampoules or as starter cultures. The former contain >5 x 10^5 cells; the latter are initiated by Lonza and sent in a T75 flask containing ~2 x 10^6 cells.

To order frozen ampoules + media:

Name:	HSMM– Normal Human Skeletal Muscle Myoblasts
Item #:	CC-2580 (HSMM - Cryopreserved ampoule)
	CC-3245 (SkGM-2™ BulletKit® = CC-3244 + CC-3246)

To order starter cultures + media:

Name:	HSMM– Normal Human Skeletal Muscle Myoblasts
Item #:	CC-2580T75 (HSMM – in SkGM-2 T-75 flask)
	CC-3245 (SkGM-2™ BulletKit® = CC-3244 + CC-3246)

Notes:

The number of BulletKits purchased depends on the target number of cells to be generated. A rule of thumb is 10 BulletKits for every initial T225 flask of cells. It is strongly recommended to purchase all of the media that will be required for a complete expansion series, since media supply may be erratic.

Materials List

1. Cell-type specific medium (BulletKits – Lonza Biosciences)
2. T75, T225 tissue culture flasks
3. Corning conical centrifuge tubes (15mL and 50mL)
4. Graduated pipets (1, 5, 10, 25, 50mL)
5. DMEM/F-12 (1:1) Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (Ham) 1X, + L-Glutamine, +15mM Hepes, - Phenol Red (Invitrogen, Cat# 11039-021)
6. Horse Serum (Lonza, Cat# 14-403E)
7. Penicillin-Streptomycin Solution (200X) (Cellgro, Cat# 30-001-CI)
8. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
9. Accutase Enzyme Cell Detachment Medium (EBiosciences Cat# 00-4555)
10. Eppendorf Centrifuge 5810R
11. Hemocytometer
12. Micropipet w/ P20 tips
13. Microscope

Growth Medium for HSMM

Skeletal Muscle Myoblast Cell Growth Medium BulletKit-2 (SkGM-2) = SkBM-2 Basal Medium (Lonza Cat# CC-3246) + SkGM-2 SingleQuots Supplements and Growth Factors (Lonza Cat# CC-3244)

Differentiation/Fusion Medium for HSMM

DMEM/F-12 (1:1) Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (Ham) 1X, + L-Glutamine, +15mM Hepes, - Phenol Red
2% Horse Serum
Pen-Strep (1X)

Procedure

A. Initiation of culture from cryopreserved cells

1) Rapidly thaw cells by holding vial and rotating in a 37^{o}C water bath.
2) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then dispense contents of the vial into a flask at **3,500 cells/cm^2 density**.
3) Let cells recover for 16 hours in a 37^{o}C, 5% CO_2 humidified incubator.
4) The next morning, the diluted DMSO-containing shipping/cryopreservation medium is aspirated from the cell layer and replaced with fresh medium.

B. Sub-culture

1) Propagate cells until density reaches 60-80% confluence.
2) Aspirate medium.
3) Wash cells with warm 1X PBS.
4) Add 15mL of Accutase and return to incubator for 10-15 minutes, or until cells detach.
5) Immediately remove cells, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4^{o}C).
6) Gently re-suspend cell pellet in warm medium.
7) Count cells with hemocytometer.
8) Add warmed medium to flasks.
9) Seed flasks at **3,500 cells/cm^2 density.**
10) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and every OTHER day thereafter.
2) Increase media volume as confluency increases (volumes assume the use of T225 flasks):
    a. 25% = 1mL/5 cm^2
    b. 25-45% = 1.5mL/5 cm^2
    c. 45%+ = 2mL/5 cm^2.
3) Per the above an exemplary schedule might be:
    a. day 1, plate into T225: use 50mL of media.
    b. day 2, change media, use 50mL of media.
    c. day 4, change media, use 100mL of media (if confluency is >50%).
    d. day 6, change media, use 100mL of media (or harvest if ready).

e. day 7 or 8 (harvest when cells reach 6 x 10^6 cells/flask).

D. Harvest

1) Pass cells 3-4 times until the desired cell number is achieved (primary cells will senesce after 4-5 passages).
2) Remove cells from flasks according to protocol described above under "Sub-culture."
3) Examine viability using Trypan blue staining (SOP TP-7).

E. Differentiation/Fusion to Multinucleated Myotubes

1) Propagate cells until desired number of cells is reached and the density reaches 50%-70% confluence.
2) Remove SkGM-2 growth medium and add differentiation/fusion medium.
3) Continue to culture the cells in differentiation/fusion medium, replacing the fusion medium every other day for 5 days, until myotubes are observed throughout the culture.
4) The resulting differentiated cultures can be observed to contain multinucleated (more than 3 nuclei) myotubes.