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Document # INST-SUP-CC-2580-4 08/12
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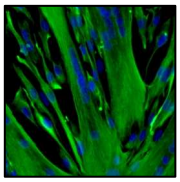

Clonetics™ Skeletal Muscle Myoblast Cell Differentiation to Form Myotubes

HSMM Differentiation – Supplemental Instructions for Use

Introduction

Myoblasts usually begin to form multinucleated myotubes when the culture reaches 50-70% confluence. Myotubes are formed by the fusion of several to many myoblasts. Addition of differentiation medium, when the culture reaches approximately 50% confluence, will promote the maturation of myotubes. The procedure hereafter describes how to differentiate the human skeletal muscle myoblasts (HSMM) using a differentiation medium, also referred to as a fusion medium.

Differentiation Components (Sold Separately)

- One skeletal muscle myoblast cell product – normal or diseased (cryopreserved or proliferating)
- One Skeletal Muscle Myoblast Cell Media BulletKit™ Medium - 500 ml

  Clonetics™ SkGM™-2 BulletKit™ (CC-3245) contains 500 ml of Skeletal Muscle Basal Medium-2 (SkBM™-2 Medium) and the following growth supplements: human Epidermal Growth Factor (hEGF), 0.5 ml; Dexamethasone, 0.5 ml; L-glutamine, 10.0 ml; Fetal Bovine Serum (FBS), 50.0 ml; Gentamicin/Amphotericin-B (GA), 0.5 ml.
- DMEM:F-12 - 500 ml (Lonza Catalog no. 12-719F or similar)
- Horse Serum - 100 ml (Lonza Catalog no. 14-403E or similar)

Initiation of Differentiation Process

1. Culture skeletal muscle myoblasts under standard culturing conditions in growth medium (SkGM™-2 Medium) until culture has achieved 50%-60% confluence.
2. Remove the growth medium and replace with an equal volume of fusion medium (DMEM-F12 supplemented with 2% horse serum).
3. Continue to culture the cells in the fusion medium (replacing the fusion medium every other day) for ~3 to 5 days, or until myotubes are observed throughout the culture. The resulting differentiated cultures can be observed to contain multinucleated (more than 3 nuclei) myotubes.
4. If the myotubes are to be used in assays that require an extended period in culture, following differentiation, remove the fusion medium and add growth medium. For best performance, replace the growth medium every other day to maintain the cultures for ~2 to 3 weeks post differentiation. Myotube cultures are best used by 2 weeks post differentiation.

Comments on Multinucleated Myotubes

(Differentiated Skeletal Muscle Cultures)

1. Not all myoblasts will fuse to form myotubes at the same time. The culture is not synchronized; however, 3 to 5 days should be sufficient to complete the fusion in nearly all of the culture.
2. Some differentiation may be observed when cultures reach ~50% confluence, even before fusion medium is added.
3. The fusion of myoblasts to form myotubes is a terminal differentiation process.
4. Some myoblasts may never fuse and will remain as myoblasts for the duration of the fusion medium treatment.
5. Differentiation is not reversed by replacing fusion medium with growth medium.
6. The above differentiation protocol can also be applied to Lonza's Skeletal Muscle Cells (SkMC) by substituting SkGM™ Medium as the growth medium instead of SkGM™-2 Medium, however, differentiation rate of Lonza's Skeletal Muscle Cells is typically significantly less than differentiation rate of Lonza's Human Skeletal Muscle Myoblasts (HSMM)

Characterization of Cells

Routine characterization of HSMM includes positive immunofluorescence staining for Desmin (≥60% positive) following differentiation in fusion medium in first passage out of cryopreservation.

Quality Control

All cells are performance assayed and test negative for HIV-1, mycoplasma, Hepatitis-B, Hepatitis-C, bacteria, yeast and fungi. Cell viability, cell number, and proliferative capacity are measured after recovery from cryopreservation. Clonetics™ Media are formulated for optimal growth of specific types of normal human cells. Certificates of Analysis (COA) for each cell strain are shipped with each order. COAs for all other products are available upon request.

Ordering Information

Cryopreserved Skeletal Muscle Myoblast Cells (Single Donor):

Cat. No.	Product	Description
CC-2580	Normal HSMM	≥500,000 cells
CC-2900	HSMM (Diabetes Type I)	≥500,000 cells
CC-2901	HSMM (Diabetes Type II)	≥500,000 cells

Proliferating Skeletal Muscle Myoblast Cells (Single Donor):

Cat. No.	Product	Description
CC-2580T25	Normal HSMM (T-25)	Proliferating, Normal HSMM in a T-25 flask
CC-2580T75	Normal HSMM (T-75)	Proliferating, Normal HSMM in a T-75 flask
CC-2580W96	Normal HSMM (96-well plate)	Proliferating, Normal HSMM in a 96-well plate
Other proliferating formats are available. Refer to the Lonza website or contact Scientific Support for details.		

Skeletal Muscle Myoblast Growth Media
(Sold Separately):

Cat. No.	Product	Description
CC-3245	SkGM™-2 BulletKit™ Medium	500 ml SkBM™-2 Basal Medium plus CC-3244 SingleQuots™ Kit to formulate SkGM™-2 Medium (growth medium)
CC-3246	SkBM™-2 Basal Medium	Skeletal muscle myoblast basal medium-2 (500 ml)
CC-3244	SkGM™-2 SingleQuots™ Kit	Formulates 500 ml of SkBM™-2 Basal Medium to SkGM™-2 Growth Medium; contains hEGF, 0.5 ml; Dexamethasone, 0.5 ml; L-glutamine, 10.0 ml; FBS, 50.0 ml; GA, 0.5 ml.

Skeletal Muscle Myoblast Fusion Media
(Sold Separately):

Cat. No.	Product	Description
12-719F	DMEM:F-12, 1:1 Mixture	500 ml
14-403E	Horse Serum	100 ml

Product Warranty

Cultures have a finite lifespan *in vitro.*

Lonza guarantees the performance of its cells in the following manner only if Clonetics™ Media and Reagents are used exclusively and the recommend protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete cell system.

1. Clonetics™ HSMM Cryopreserved Cultures are assured for experimental use for 10 population doublings. Clonetics™ HSMM from Diabetic Donors Cryopreserved Cultures are tested for two passages for population doublings FIO (For Information Only).
2. Clonetics™ HSMM Proliferating Cultures and HSMM from Diabetic Donors Proliferating Cultures are assured for experimental use for one passage upon receipt.
3. Additional population doublings and subcultures are possible, but growth rate, biological responsiveness and function deteriorate with subsequent passage.
4. To avoid the loss of your cells and forfeiture of your warranty, subculture cells before they reach 50-70% confluence.

When placing an order or for Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics™ Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or want to speak with Scientific Support, you may contact Lonza by web, e-mail, telephone, fax or mail
(See page 1 for details).

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th ed. If you require further information, please contact your site safety officer or Scientific Support.