# MLPC™-UCB Clonal Cell Line
## Myogenic Differentiation of MLPC

### Reagents and Materials

- MSCGM, Lonza Walkersville, Inc., PT-3001
- Skeletal Muscle Growth Media Kit, Lonza Walkersville, Inc., CC-3160
- FGF-basic, Sigma, F-0291
- 5'-Azacytidine, Sigma, A1287
- Cardiogenol C, Sigma, C4866
- PBS, Gibco, 21600-069 or equivalent
- Fibronectin biocoated chamber slides, Becton Dickinson Labware, 354559
- Hank's Balanced Salt Solution, Sigma, H1641 or equivalent
- Bovine Serum Albumin, Fisher, BP1600-1 or equivalent
- Paraformaldehyde, Sigma, P-6148 or equivalent
- Triton X-100, Sigma, X100 or equivalent
- anti-fast skeletal myosin [MY-32], Abcam, ab7784
- anti-alpha actinin [BM 75.2], Abcam, ab11008
- anti-atrial natriuretic peptide [M622709], Abcam, ab2093
- anti-cardiac troponin I [19C7], Abcam, ab8289
- anti-cardiac troponin T [1C11], Abcam, ab8295
- Alexa Fluor 594 GAM, Molecular Probes, A11020

### Skeletal Myocyte Procedure

1. Plate MLPCs in fibronectin coated 4-well chamber slides at $1.9 \times 10^4$ cells/well in MSCGM.
2. Incubate the cells in a 5% $CO_2$ atmosphere at 37°C.
3. Cells are fed every 3-4 days with MSCGM until the cultures reach confluency.
4. At confluency, medium is removed and replaced with 10µM 5'-azacytidine in MSCGM and incubated for 24 hours.
5. Cells are washed with PBS and fed with SkGM Skeletal Muscle Growth Medium supplemented with FGF-basic.
6. Cultures are harvested 14 days post initiation of myogenic culture.
7. Cells are stained with antibody for skeletal myosin and alpha-actinin, both indicative of MLPC differentiation into skeletal muscle cells.

### Cardiomyocyte Procedure

1. Plate MLPCs in fibronectin coated 4-well chamber slides at $1.9 \times 10^4$ cells/well in MSCGM.
2. Incubate the cells in a 5% $CO_2$ atmosphere at 37°C.
3. Cells are fed very 3-4 days with MSCGM until the cultures reach confluency.
4. At confluency, meduim is removed and replaced with Skeletal Muscle Growth Medium supplemented with 100 ng/mL FGF-basic, 10µM 5'-azacytidine and 10µM Cardiogenol C in the experimental wells. The control wells are continued to be fed with MSCGM.
5. Continue to feed every 3-4 days with the above medias.
6. Although no beating myocytes were observed, well formed myofibers were observed as well as the development of several proteins associated with cardiac tissue after 21 days in culture with SkGM.

### Staining Procedure

1. Remove the medium from each well of the chamber slide.
2. Wash each well twice with 1 mL Hank's Balanced Salt Solution-HBSS (with $Ca^{+2}$ and $Mg^{+2}$) supplemented with 1% BSA .
3. Add 1mL of 4% Paraformaldehyde to each well.
4. Incubate for 20 minutes at room temperature.
5. After incubation, remove the 4% Paraformaldehyde and wash the wells twice with 1 mL of HBSS + 1% BSA.
6. Add 1 mL of 0.5% Triton X-100 to each well.
7. Incubate for 3-5 minutes at room temperature.
8. After the incubation, wash each well twice as directed above.
9. Add approximately 500µl of HBSS + 1% BSA to each well.
10. Add the appropriate amount of primary antibody (manufacturer's recommended dose) to the appropriate well and incubate for 20-30 minutes.
11. If primary antibody is fluorescently labeled, cells are washed twice with HBSS + 1% BSA. The chamber slide is removed. Cover the slide with a coverslip, retaining some fluid underneath to keep cells moist while examining under a fluorescent microscope.
12. If the primary antibody is unlabeled, the cells are washed twice with HBSS + 1% BSA then Alexa Fluor 594 GAM is added. Cells are incubated with the secondary antibody for 20 minutes at room temperature.
13. Cells are washed twice with HBSS + 1% BSA. The chamber slide is removed. Cover the slide with a coverslip, retaining some fluid underneath to keep cells moist while examining under fluorescent microscope.