Scope

Muscle Progenitor cells have been selected based on differential adhesion. The cell culture protocols described in this manual include the *in-vitro* culture of human skeletal muscle progenitor cells using optimized reagents. Any changes in the experimental conditions may have negative effects on cell survival and may yield abnormal cell growth.

Materials and Equipment

Materials

- Muscle Progenitor Cells (AM002-F)
- Muscle Cellutions Medium (M-GRO)
- Muscle Cellutions Differentiation Medium (M-DIFF)
- FGF-2 (20 ng/ml, concentration in M-GRO, to be added – not supplied)
- Collagen coating solution (150 µg/mL)
- 1× PBS (Ca^{2+}/Mg^{2+} free)
- Fetal bovine serum (FBS)
- TrypLE Express or equivalent enzyme solution
- 15.0 ml and 50.0 ml conical centrifuge tubes
- Tissue culture vessels
- Dimethyl Sulfoxide (DMSO), if freezing cells

Equipment

- Biosafety Cabinet
- Water Bath, 37°C
- Centrifuge
- Incubator, 37°C, 5% CO_2
- Pipet-Aid
- Pipettes

Procedure

1.	Immediately upon delivery • Remove vial from shipping container and check that it is still frozen—if thawed, contact DV Biologics. • Transfer frozen vial immediately to liquid nitrogen until you are ready to thaw and begin cell culture. • Prior to thawing cells, make sure all reagents are ready to use. • Coat cell culture vessels with collagen coating solution and incubate at room temperature, 1h. • Aspirate collagen coating. • Wash the culture vessel with PBS.
2.	Thawing Cells • Perform and maintain all cell culture using aseptic techniques. • Prepare M-GRO medium. After mixing, store complete medium at 4°C for a maximum of 30 days. • Aliquot and warm only media required. To the M-GRO for use that day, add fresh FGF-2 (20ng/ml). • Thaw cells rapidly and with agitation (See How to Thaw Cells). ○ Slowly dilute thawed cells with 3- to 10-fold excess cold FBS (or complete M-GRO medium). ○ Centrifuge at 400 g for 5 minutes. • Remove supernatant and disperse cell pellet in fresh 37°C warm M-GRO medium with FGF-2. • Count cells (see How to Count Fresh Cells).
3.	Plating and Maintaining Cells • Carefully add cell suspension to **culture vessel prepared with collagen coating solution**. Seed cells at a density of 5.0 x 10^3 cells/cm^2 -1.0 x 10^4 cells/cm^2. • Leave cells undisturbed for 2 days. Feed cells on the third day by completely replacing the medium. NOTE: There may be many unattached cells. These cells could be progenitors or stem cells that have not yet attached. You may collect these, centrifuge and reseed. • Feed cells every 2-3 days with fresh M-GRO medium (with FGF-2 added) by either: ○ Performing a complete media replacement and discarding the spent media, or

	○ Removing the spent media but not discarding until after collecting any unattached cells by centrifugation as noted above. After centrifuging, aspirate supernatant and disperse cell pellet in M-GRO medium with FGF-2 to reseed. • Once cells become 60-70% confluent, feed more regularly (every other day). • When cells are nearing confluency (approximately 80%), prepare to passage cells either for propagation/expansion of the progenitor cells or for their terminal differentiation. NOTE: Do not let cells become overly confluent.
4.	Passaging Cells (for Details see How to Passage Cells) • Aspirate and discard medium; wash cells with PBS without Ca^{2+}/Mg^{2+}, and aspirate. • Add TrypLE Express or equivalent enzyme solution at a volume that just covers the cell monolayer. • Incubate at 37°C; microscopically observe detachment of cells every 5 min; avoid toxic effects of enzymatic over-digestion. • Quench the enzyme digestion by adding 2- to 5-fold volume of M-GRO medium. • Transfer the entire resulting suspension of detached cells from the culture vessel to a centrifuge tube. • Centrifuge the cell suspension at 400 g at 4°C for 5 min. • Aspirate supernatant and disperse cell pellet in warm M-GRO medium for counting. Count cells (see How to Count Fresh Cells). • To propagate muscle progenitor cells, seed cells at 1.0 x 10^4 cells/cm^2 following the differential adhesion/pre-plate method described in the next section. • If more cells are obtained than needed for re-plating, additional cells may be frozen. (See How to Freeze Cells).
5.	Differential Adhesion/Pre-Plate Method • Coat flask with collagen and set aside until time for second seeding of cells. • For the first seeding of cells, plate cells on **NON-coated** flasks at a density of 1.0 x 10^4 cells/cm^2 in M-GRO medium. • Incubate for 30 minutes in 37°, 5% CO^2 incubator. • Transfer medium and non-adhered cells to a tube. • Rinse flask with fresh M-GRO medium and pool rinse with non-adhered cells. • Centrifuge at 400 g for 5 minutes at 4°C. • Aspirate supernatant and disperse cell pellet in M-GRO medium for counting. Count cells (see How to Count Fresh Cells). • Seed cells into **collagen-coated** flask at a density of 1×10^3 to 5×10^3 cells/cm^2. • The non-coated flask, which has a mix of some fibroblasts and muscle cells, can be discarded. • Repeat these steps for each passage until ready to differentiate. • For terminal differentiation use p3 to p5 cells.
6.	Terminal Differentiation • Seed cells in flask/wells using **M-GRO medium (with FGF-2 added),** prepared as previously noted. • Once confluent, feed cells with **50/50 blend of M-GRO (without FGF-2) and M-DIFF**. • Let cells acclimate to new medium for 1 day. This will be Day 0. • On Day 1, feed cells with a complete medium replacement using **M-DIFF medium**. • Repeat the above step for Day 2 and Day 3, feeding cells each time by replacing with fresh M-DIFF medium. NOTE: Observe cells regularly for lifting and toxicity. • Maintain cells in M-DIFF medium from Day 4 onward for a minimum of 2 weeks, with fresh M-DIFF medium replacement every 3 to 4 days.