Culture Instruction Manual & Protocols

Materials & Reagents Required

1X PBS (Ca2+/Mg2+-free)
Coating matrix: Collagen
Trypsin or equivalent
Muscle Cellutions Medium (Ref: P60147)
Muscle Cellutions Differentiation Medium (Ref: P60148)
Dimethyl Sulfoxide (DMSO)
bFGF (10 ng/ml)
T225 flask,
6 or 12 well plates
Incubator, 37 °C/5% CO2.
Tissue culture vessels
Water Bath, 37 °C
15.0 & 50.0 mL tubes.
Centrifuge
Pipette
Ice
Not Provided: Cell Culture Media; Differentiation Media; Growth Factors

* These cells have been selected for by differential adhesion. The cell culture protocols described in this manual include the in-vitro culture of human skeletal muscle progenitor cells (myoblasts) using optimized reagents. Any changes in the experimental conditions may have negative effects on cell survival and may yield abnormal cell growth. For more information and for a complete list of Innoprot’s reagents and products contact our customer service.

General Considerations: The protocols included in this manual are intended to serve as a guide only, and optimization of culture protocols is encouraged to ensure success.
1.0 IMMEDIATELY UPON DELIVERY

1.1 Remove vial from shipping container to check that it is still frozen.

1.2 Transfer frozen vial to liquid nitrogen until you are ready to thaw and begin cell culture.

2.0 THAWING CELLS

2.1 Prior to thawing cells, coat cell culture vessels with collagen for immediate use/propagation of progenitor cells.

2.2 Thaw cells rapidly. Do not allow sample to warm 37ºC. Cryovials should be cool to the touch when removed from bath. Passive thaw is not recommended

*Note: Make sure to wear safety glasses, gloves and a face shield*

**CAUTION:** If thawing in a 37ºC water bath there should be a small amount of ice left over. Cell viability will be reduced if allowed to remain in freezing media after thawing. Therefore, do not allow the cells to remain in freezing media.

2.3 Immediately transfer to a 15 mL tube (work quickly)

2.4 Drop wise add FBS at a rate of 1 minute per ml until the 8 ml demarcation. Swirl the tube gently to mix contents.

2.5 Centrifuge at 300 x g for 5 minutes. Remove supernatant and resuspend cell pellet in fresh 37°C warm medium.

2.6 Place cells into collagen coated culture vessels. Seed cells at 5,000-10,000 cells/cm².

**Important:** Leave cells undisturbed for a minimum of 2 days

3.0 CULTURE/MAINTENANCE FOR CELLS

3.1 Feed cells every 3 days by completely removing the media.

*Note: There will be a lot of cells unattached. These cells may be progenitors or stem cells that have not yet attached. You may collect these and reseed*

3.2 Observe cells on a daily basis.

3.3 Once cells become 60-70% confluent, feed more regularly (every other day).
| 3.4 | Once cells get confluent prepare vessels for passage/expansion  
**Important**: Do not let cells get over confluent |
| 4.0 **PASSAGING OF CELLS** | |
| 4.1 | Aspirate and discard medium; wash cells with warm (37°C) PBS, aspirate, and add enzyme solution |
| 4.2 | Incubate for 5 min at 37°C.  
**Important**: Observe detachment of cells. When using trypsin observe the detachment of cells every 1-2 minutes in order to avoid toxic effects |
| 4.3 | Add FBS immediately to detached cell suspension to stop reaction. Transfer cell suspension to a desired centrifuge tube |
| 4.4 | Wash cell culture vessel by adding warm PBS to the culture vessel to assure you have obtained all cells. Add the washed material to centrifuge tube used in step 5.2. |
| 4.5 | Centrifuge your cell suspension at 300 x g for 5 minutes |
| 4.6 | Aspirate supernatant and resuspend cells in fresh media for counting |
| 4.7 | Seed cells in precoated matrigel cell culture vessels at 5,000-10,000 cells/cm² |
| 5.0 **TERMINAL DIFFERENTIATION** | |
| 5.1 | Seed cells in flask/wells |
| 5.2 | Once confluent, transition your cells to differentiation medium by using a 50/50 blend of M- Gro-001 (growth medium) and M-Diff-001 (terminal differentiation medium). Remove the use of bFGF from your medium.  
**Important**: Let cells acclimate to new media for a minimum of 1 day |
| 5.3 | The following day do a complete medium change using M-Diff-001 terminal differentiation medium |
| 5.4 | Feed cells every 2-3 days by completely removing the medium followed by a warm PBS wash.  
**Important**: Observe cells regularly for lifting and toxicity. |
| 5.5 | Leave cells in terminal differentiation medium for a minimum of 2 weeks |