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# C2C12 Myoblast Growth and Differentiation

## Growth:

1. Thaw frozen vial of C2C12 myoblasts and transfer to conical tube containing C2 growth media (recipe below).
2. Spin at ~300xg (1200 RPM in Eppendorf 5410 centrifuge) for 5' to pellet cells.
3. Aspirate and resuspend in C2 growth media. Transfer to tissue culture flasks or plates and grow at 37°C, 5% $CO_2$.
4. Subculture every 1-3 days, making sure that the cells never grow to more than 50% confluence. This is typically achieved by splitting the culture 1:10. If cells become crowded, they can spontaneously differentiate, and thus continued passaging of the cells will propagate cells with limited differentiation capacity. It is generally best to use as cells that have not been continuously passaged by freezing down many vials of cells at the earliest passages.

## Differentiation:

Note:The best results for long-term culture of differentiated C2C12 cells can be achieved by plating myoblasts on matrigel coated tissue culture plates. This is done by diluting Matrigel (B-D Biosciences) 1:30 in cold PBS and putting enough on to cover the surface of the plates. Let this sit overnight at 4 degrees, or if you are in a hurry, a few hours at room temperature. Matrigel is expensive and should be used sparingly. When thawing, it is best to thaw it on ice or in the fridge slowly, avoiding warming it with your hands. If Matrigel is thawed too quickly, it will form insoluble clumps. If you are not planning on culturing differentiated C2C12 cells for a long time, it is fine to proceed with the differentiation protocol using uncoated dishes.

1. Plate C2C12 cells at a density of 5,000-10,000 cells/$cm^2$ in standard C2 growth media.
2. The next day, the plate should be 30-50% confluent. Aspirate the growth media, wash the cells 1x with PBS, and replace with C2 differentiation media.
3. Cells will begin to differentiate within 24-48 hours, though fusion into myotubes will typically take 4-6 days. Replace the differentiation media every 3-4 days.

C2 Growth media: (DMEM 15% FBS)
207 ml DMEM high glucose (Invitrogen 11960-044)
37.5 ml Fetal Bovine Serum
2.5 ml L-Glutamine solution (Invitrogen, 100x stock)
2.5 ml Penicillin-Streptomycin solution (Invitrogen, 100X stock)
0.5 ml Primocin (Invivogen, 500x stock)
250 ml total volume
0.2 µm filter and store at 4°C for up to a month.

C2 Differentiation media: (DMEM 0.5% FBS + ITS)
207 ml DMEM high glucose (Invitrogen 11960-044)
1.25 ml Fetal Bovine Serum
2.5 ml Insulin-Transferrin-Selenium Supplement (ITS, Invitrogen, 100x stock)
2.5 ml L-Glutamine solution (Invitrogen, 100x stock)
2.5 ml Penicillin-Streptomycin solution (Invitrogen, 100X stock)
0.5 ml Primocin (Invivogen, 500x stock)
250 ml total volume
0.2 µm filter and store at 4°C for up to a month.