

## Culture, Differentiation and Transfection of C2C12 Myoblasts

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**[Abstract]** C2C12 myoblasts are commonly used in biomedical laboratories as an *in vitro* system to study muscle development and differentiation. This protocol explains the basic procedures of culture, transfection and differentiation of C2C12 myoblast cells.

### **Materials and Reagents**

1. C2C12 myoblasts
2. DMSO (Sigma-Aldrich, catalog number: 472301)
3. Fetal bovine serum (FBS)
4. Horse serum
5. DMEM (high glucose) (Life Technologies, Invitrogen™, catalog number: 11965142)
6. P/S solution
7. Fugene HD (FHD) (Roche Diagnostics, catalog number: 04709691001)
8. Growth media (see Recipes)
9. Transfection mix (see Recipes)
10. Freezing media (see Recipes)
11. Differentiation media (see Recipes)

### **Equipment**

1. Standard tabletop centrifuges
2. Water bath
3. CO<sub>2</sub> incubator
4. 100 mm culture dishes
5. Eppendorf tube

### **Procedure**

#### A. Grow cells from frozen stock

1. Briefly thaw cells in a 37 °C pre-warmed water bath.

2. Once cells are thawed, pipette into Eppendorf tube and spin for 5 min at 1,000 rpm. Aspirate media. Resuspend cells in 10 ml growth media and plate in 100 mm dish.
3. Split the cells when they grow to 80% confluency.
4. Refreeze the cells: freeze the cells in freezing media.

#### B. Passage cells

1. Once cells reach 80% confluency, split as 1:40 to a new dish. 3-4 days later, it will be 80% confluency again.
2. Never let cells grow confluency. They will differentiate.

#### C. Transfection

1. Day 0: seed cells (low density, < 50%).
2. Day 1: transfection: (optimum 20% FBS, NO P/S).
3. Day 2: change media (growth media for regular growth or differentiation media for differentiation purpose).
4. 4-5 days for complete differentiation under confluency.
5. 3-4 days for complete differentiation under starvation media, but growth is restricted.

Example:

1. For 6-well plate: seed 100,000-150,000 cells total in all 6 plates (10-15,000/cm<sup>2</sup>).
2. Transfect 1 µg DNA/6-wells (total).

#### Recipes

1. Freezing media
  - 50% FBS
  - 10% DMSO
  - 40% Growth media
2. Growth media for C2C12 cells
  - DMEM
  - 20% FBS
  - 1% P/S
3. Transfection mix
  - FHD (1 µg DNA: 4 µl FHD)
4. Differentiation media
  - DMEM
  - 1% horse serum
  - 1% P/S

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