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_2 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^2).
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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