

C2C12 myogenic differentiation protocol

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1. Grow myoblasts in 100 mm plates to 50% confluence.
2. Maintain cells at 37°C and 10% CO₂ in growth medium (Dulbecco's modified Eagle's medium (DMEM), supplemented with 20% fetal bovine serum [FBS] and 1% penicillin-streptomycin antibiotic.
3. Change media every 24-36 hrs.
4. Induce differentiation into myotubes by transferring myoblasts into differentiation medium consisting of DMEM with 2% horse serum and 1% penicillin-streptomycin.
5. Continue incubation at 37°C and 10% CO₂
6. Change differentiation media every 24 hrs.
7. Visualization under light microscopy should reveal myotube formation after 24 hrs in differentiation medium.