Frequently Asked Questions about Myoblast Cell Cultures

1. **What growth medium is used to cutlure myoblast cell lines?**

   15% (U) FBS in HF10 + 10ng/ml basic fibroblast growth factor (bFGF)

   1) Nutrient Mixture Ham's F10 (HF10, Invitrogen 12390-035 or equivalent)
   2) Fetal Bovine Serum – uninactivated (Sigma or Hyclone)
   3) Basic Fibroblast Growth Factor (bFGF, Peprotech #100-18B or equivalent)
      a. Place the vial containing the basic fibroblast growth factor (bFGF) in a microcentrifuge with an empty vial opposite to balance. Start the centrifuge and allow the speed to reach 10,000 rpm, then stop the run. NOTE: There may be no visible material in the vial.
      b. Prepare 0.2% BSA in 5mM Tris•HCl by adding 0.3g BSA to 150ml 5mM Tris•HCl, pH 7.5, while stirring on a magnetic stir plate until dissolved, then sterilize by filtration.
      c. Remove the bFGF vial to the biosafety cabinet and wipe the outside with a 70% alcohol swab. For a 50 µg vial, add 0.5ml of sterile 5mM Tris•HCl and pipet gently to dissolve. Transfer the solution to a 15 ml centrifuge tube, rinse the vial with another 0.5ml of 5mM Tris•HCl, and add this to the tube.
      d. Dilute the 1ml bFGF solution to 5ml with sterile 5mM Tris•HCl containing 0.2% bovine serum albumin (i.e., add 4 ml) to yield 10 g/ml bFGF (1000X).
      e. Filter sterilize using a 10cc syringe and 0.2µm pore 13mm diameter filter.
      f. Dispense 0.5ml aliquots into labeled, sterile 1.5ml microcentrifuge tubes and store in the -80°C or –20ºC freezer.

2. **What trypsinization solutions will be needed to subculture myoblast cell lines?**

   1) EDTA solution: Versene 1:5000 (Invitrogen #15040-066 or equivalent)
   2) 0.05% Trypsin/ 0.53mM EDTA (Invitrogen #25300-054 or equivalent)

3. **Is it recommended to us a culturing substrate?**

   Yes, either 1.5µg/cm^2 fibronectin or gelatin may be used.

   1) Human plasma fibronectin (Millipore 1mg/ml solution #FC010-10MG or equivalent). Dilute stock to 12.5 µg /ml and add 3ml per 25cm^2 flask. Incubate flasks in a horizontal position in a humidified 5% CO_2-air incubator at 37°C for 30-60 minutes for immediate use *or* store flasks at 4°C overnight (cold flasks can be kept for up to 2 months).
   2) 1% gelatin (Sigma-Aldrich, St. Louis, MO #G1890) in 0.9% sodium chloride. Dissolve and sterilize by autoclaving at 121 °C / 90 min. Store at room temperature. Add 3ml per 25cm^2 flask and incubate at 37 °C as described for fibronectin above.

4. **How is a myoblast cell line subcultured?**

   Volumes are for 25cm^2 flasks

   1) Culture must be subcultivated **prior to** confluence or myoblasts will begin to fuse.
   2) Remove medium.
   3) Rinse cell monolayer with 3 ml of EDTA solution.
   4) Remove EDTA solution.
   5) Add 1 ml of Trypsin – EDTA solution to flask for 2-7 minutes or until cells start to come off flask.

6) Add 5 ml of growth medium to flask. Gently mix cells and medium by trituration. Remove 0.5ml to count.
7) Aspirate coating solution from new flask and inoculate flasks at (0.2 – 2) x 10^4 cells/cm^2.
8) Incubate flasks at 37°C in a 5% CO_2 incubator. Cultures plated at the higher density of 2 x 10^4 cells/cm^2 will typically need to be split or harvested within 3 - 5 days.

5. What is the freezing medium used to cryopreserve myoblast cell lines?

Growth medium + 15% FBS + 5% DMSO

6. How should myoblast cell lines be cryopreserved and stored?

1. Place cells into a single-cell suspension, count and pellet as indicated in the subculture protocol above.
2. Resuspend the cells in freezing medium to a seeding density of 5.0e5 viable cells per ml
3. Aliquot 1 ml into each cryovial or ampule.
4. Cells resuspended in freezing medium should be immediately placed in a controlled rate freeze machine that reduces temperature at a controlled rate of -1°C/min. Alternatively, cryovials can be placed in an ethanol bath at -80°C overnight before being placed in liquid nitrogen vapor.
5. Frozen cell stocks are stored in liquid nitrogen tanks. Glass ampules are submerged in liquid, plastic cryovials are stored in vapor phase.

*Suppliers of reagents are listed for the convenience of culture recipients only. Such lists are not intended to be either selective or exhaustive, and Coriell Institute does not recommend specific products or suppliers. Other media and reagents may be satisfactory, but have not been tested.