Week 2: Bovine Satellite Cell Isolation (Muscle)

Jan 25th, 2024

General Notes:

* This is the muscle cell isolation – other groups will perform isolations for fat cells for the class

Materials:

* *Tools*:
  + scalpel,
  + tweezers
  + petri dishes
  + flasks
  + 70 um cell strainers
  + 40 um cell strainers
* *Reagents:*
  + DPBS + 10% Anti/Anti (prepared Week 1)
  + Growth Media (prepared Week 1)
  + DMEM + Glutamax
  + 2% Collagenase solution

Protocol Day 1 – Digestion (Thursday 01.25.2024)

1. Place tissue into sterile petri dish with some DPBS + 10% Anti/Anti (~1-3 mL) to keep tissue wet
2. Mince with tweezers & scalpel, mincing into 1-3 mm3 chunks, or a thick paste
3. Move tissue to 1x 50 mL falcon tube
4. Add 18 mL DMEM
5. Add 2mL of digestion solution (2% *collagenase II*) for a final concentration of 0.2% collagenase
6. Incubate & shake tube at 37 degrees for 1 hours, triturating (pipetting up/down) every 20 mins
   1. Can incubate 30 more minutes if cells are not appropriately suspended
7. Add 20 mL of Growth Media to halt the collagenase’s activity
8. Filter cell suspensions through 70 um cell strainer into a new 50 mL tube
9. Filter again through 40 um strainers into a new 50 mL tube
10. Spin @ 400g for 10 minutes to pellet the cells
11. Carefully aspirate the supernatant without disturbing the cell pellet
12. Add 10 mL growth media and pipette up and down to resuspend the cells
13. Count cells, only counting small round cells
14. Plate ~50,000-100,000 cells / cm^2 onto uncoated plates or flasks
15. Add Growth Media to the appropriate volume for the flask
16. Label the flask with your group name, the date, and “Pre-plate”

Protocol Day 2 – Pre-plate transfer (Friday, 01.26.2024)

1. Remove supernatant from previously plated flask, and transfer to a new flask of equal size.
   1. Label with group name, date, and “BSC P0” (for “Bovine Satellite Cells, Passage 0”)
2. Add Growth Media to the Pre-Plate flask (see volume table below)
3. Add 50% extra media to the BSC P0 flask (i.e., add 2.5 mL, 6 mL, or 15 mL for a T-25, T-75, or T-175, respectively)
4. Tilt the flask back and forth slightly to mix the cells and media.
5. Set to incubate. DO NOT TOUCH for 6 days. After 6 days (e.g., Thursday February 1st), feed regularly using growth media (change media every 2-3 days).

Reference volumes:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Vessel** | **Surface (cm2)** | **~PBS volume** | **~Trypsin volume** | **~Media volume** |
| 6-well plate | 9.6 | 1 mL | 500 uL | 2 mL |
| 12-well plate | 3.5 | 500 uL | 250 uL | 1 mL |
| 24-well plate | 1.9 | 500 uL | 250 uL | 1 mL |
| 48-well plate | 1.1 | 200 uL | 100 uL | 500 uL |
| 96-well plate | 0.32 | 100 uL | 50 uL | 200 uL |
| T-25 | 25 | 3 mL | 1 mL | 5 mL |
| T-75 | 75 | 5 mL | 2 mL | 12 mL |
| T-175 | 175 | 10 mL | 3 mL | 30 mL |