Standard Operating Procedure for Cell Passaging

Jeffrey Amidon
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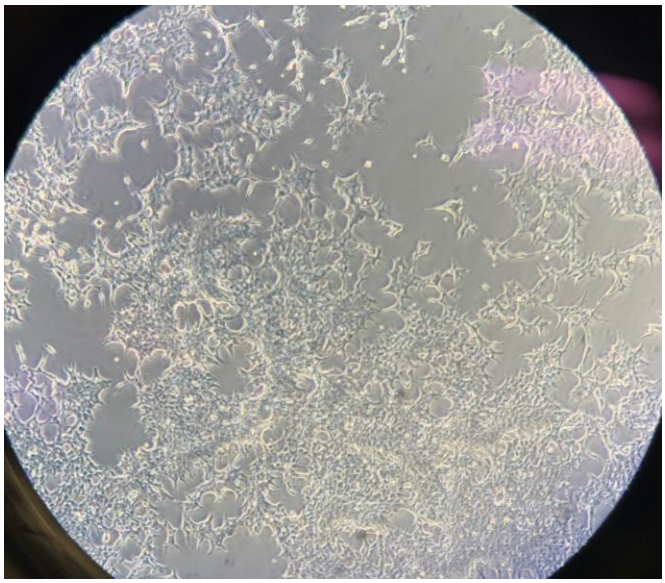
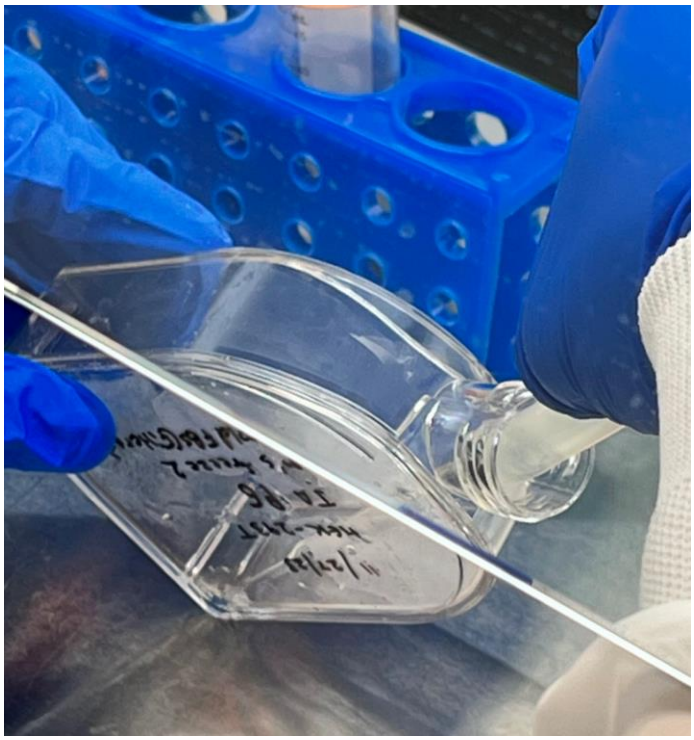
Media: 10% FBS (50 mL), 1% pen strep (5 mL), DMEM (500 mL), 2% Glutamax (10 mL of 100x) if not already in DMEM

DMEM - 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate, 200 mmol Glutamax (100ml)
Ideally buy DMEM with phenol red to easily monitor cell media acidity which indicates nutrients are mostly used up
Phenol red free media should be used for microscopy imaging

Hood and Cell prep:

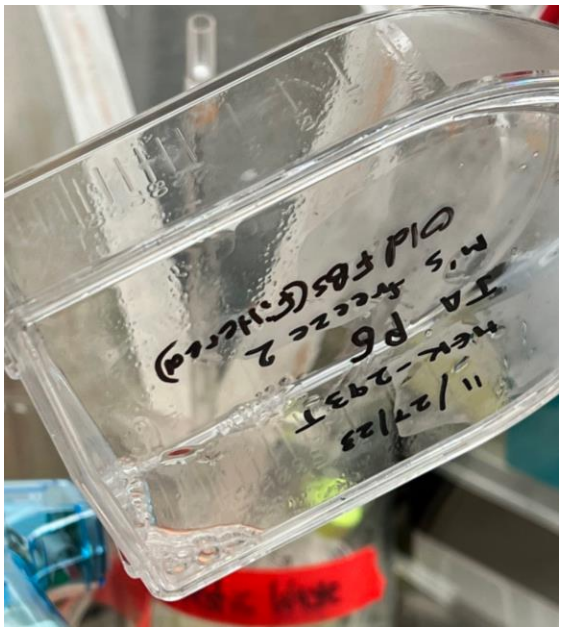
1. Cells should be roughly the following confluency (~80%)
   a. 
2. Open hood slightly and turn on blower before opening all the way
3. Spray bottom 6in of shield with 70% Ethanol and wipe down (wipe with soaked chem wipe)
   a. Treat front lip of hood in the same way
   b. Spray down working surface of hood with 70% Ethanol (wipe with soaked chem wipe)
4. Preheat media and trypsin (~20 mL) at 37°c for ~5-10 min or till warm to touch

5. Label all new culture equipment with:
    a. Cell line, Passage #, Initials, Date
6. Remove cells from incubator
7. Aspirate media off of cell culture making sure to not touch cells
    a. 
8. Treat with 3-4 ml of trypsin (for 75cm^2 flask)
    a. Let sit for 2 min or until cells start detatching
    b. Shake softly to disadhear stingy cells
    c. Suck up solution with pipette and wash bottom of flask ~5 times to ensure all cell aggregate are broken up
        i. Bottom should be clear and solution should be cloudy

ii. 

For cells tolerant of Trypsin:

9. Neutralize Trypsin with 5 ml (at least double the amount of trypsin used) of Media
10. Once neutralized, take solution volume and divide into desired fractions
    a. 4-16 fractions usually, can discard fractions if not trying to grow cells up

i. 

11. Once separated out add media ~15 ml for each 75cm^2 flask

12. Place back in incubator

For cells that require removal of Trypsin:

13. After cells are detached, pipette entire contents of flask into a 15 mL centrifuge tube.
14. Place the tube in the centrifuge and spin at setting 2 for 2 mins (make sure to have a counterbalance tube)
    a. A pellet should develop, discard supernatant and resuspend in approx 10-15 mL of media
15. Split this mixture equally amongst the new flasks
16. Place back in 5% CO_2 incubator

Hood Closure:

1. Once all work in hood is done bring in 50% bleach
2. Treat all used pipettes with bleach solution and dispose of in biohazard bin
3. Suck up ~30 ml of bleach solution into in hood vacuum to clean the line
4. Wipe down all working surfaces with 70% Ethanol
5. Close hood till roughly 1 inch above closed
6. Spray down lip and glass again (wipe with soaked chem wipe)
7. Close hood all the way
8. Turn off blower
9. Turn on UV light