## Harvest cells from antioxidant treatments

**Date: April 18, 2023**

## Objectives, Hypotheses

We will harvest the cells seeded and treated last class and store them in the fridge or at room temp until next class. The variable storage conditions will represent varying oxidative “challenges” designed to induce lipid oxidation. We expect antioxidant-treated cells to show less lipid oxidation if the cells have taken up the antioxidants during culture.

## Protocol/Procedure

Cell Harvesting

1. Aspirate media from the 3x t175 flasks
2. Add 8 mL PBS to each flask
3. Thoroughly scrape bottom of flask to release cells
4. Once cells are detached, transfer cells from the three flasks to the three 15mL tubes and label appropriately depending on which cells they contain.
5. Count the cells
	1. Mix the cells well (pipette most of volume up and down 7-10 times)
	2. Transfer 10 uL to a hemocytometer
	3. Count the four corners of the grid and perform:
		1. Cells / mL = (total count / 4) \* 10,000
		2. Total cells = (Cells / mL) \* [Volume of solution]
6. Identify your *lowest* cell number condition. For the other two conditions, remove the appropriate amount of cell suspension so that all tubes have an equal **total** number of cells.
	1. For example, suppose you have the following cell counts
		1. Flask A: 40,000 cells/mL and 320,000 total cells
		2. Flask B: 30,000 cells/mL and 240,000 total cells
		3. Flask C: 50,000 cells/mL and 400,000 total cells.
	2. The lowest cell number condition is flask B with 240,000 total cells. Thus, you will remove cell suspension from flasks A and C such that each tube also has 240,000 total cells. For flask A, we need to remove 80,000 cells. This means pipetting out 2 mL of cell suspension. For flask C, we need to remove 160,000 cells. This means pipetting out (160,000 cells / (50,000 cells/mL)) = 3.2 mL.
7. Centrifuge all cells at 300 x g for 5 mins [Make sure to balance appropriately]
8. Aspirate the supernatant, being careful not to aspirate the cells.
9. Resuspend the cells in 2 mL of PBS for each tube
10. Pipette 900uL of each condition into two labeled 1.5mL Eppendorf tubes.
	1. Each group will have two labeled 1.5mL Eppendorf tubes.
	2. Each group will have 6 tubes
		1. 2 per condition
		2. All labeled with [class meeting day, group #, condition]
11. Centrifuge all cells at 300 x g for 5 minutes
12. Aspirate the supernatant, being careful not to aspirate the cells but removing as much liquid as possible.
13. Store one tube for each condition in the fridge until next class
14. Store tube for each condition at room temperature until next class