Week 10: Thaw Muscle Cells and Antioxidant Treatments

BME-0174

April 4th, 2024

General Notes

* df = dilution factor = final volume / volume of stock transferred
	+ where final volume = volume of diluent + volume of stock
* Food-grade materials: Carrot juice, V8, or turmeric
* Lab-grade materials: Beta carotene, lycopene, or curcumin

Materials

* Bovine satellite cell growth media (BSC-GM)
* 10 mg/mL puromycin
* DMSO
* Acetone
* Beta carotene
* Carrot juice
* Lycopene
* V8 juice
* Curcumin
* Turmeric
* Filters
	+ 150 mL bottle top filters for the juices
	+ Syringe filters and syringes for the powders
* 48-well plate
* 15 mL conical tubes
* BSC cell suspension (100,000 cells/mL), prepared by instructors

Method

1. Prepare the antioxidant powders/juices
	1. NOTE: You will be assigned to one of three conditions
		1. Carrot juice & beta carotene
		2. V8 & lycopene
		3. Turmeric & curcumin
	2. Food-grade preparations (Carrot juice, V8, or turmeric)
		1. IF you are assigned [group i or ii], sterile filter the juice
			1. Add 20 mL juice to a 50 mL conical tube
			2. Spin at max speed on the centrifuge for 10 minutes
			3. Sterile filter through a 150 mL bottle top filter
		2. IF you are assigned [group iii], prepare turmeric “solution”
			1. In 15 mL tube, add 100 mg then add 9.9 mL water. In the hood, sterile filter into a labeled 15 mL tube.
	3. Lab-grade preparations: Purified carotenoids (10 mg/mL solution).
		1. [Group i] Beta carotene: In a 15 mL tube, add 15 mg then add 1.5 mL DMSO. In the hood, sterile filter into a labeled 15 mL tube.
		2. [Group ii] Lycopene: Already in solution at 10 mg/mL in acetone. Already sterile.
		3. [Group iii] Curcumin: In a 15 mL tube, add 10 mg then add 1 mL DMSO. In the hood, sterile filter into a labeled 15 mL tube.
2. Prepare media with added antioxidants.
	1. First, prepare solvent (DMSO or acetone) control at 0.1%
		1. To 15 mL of BSC-GM, add 15 µL of the appropriate solvent
	2. Prepare most concentrated lab-grade sample (10,000 ng/mL)
		1. To 10 mL of BSC-GM, add 10 µL of carotenoid powder solution
	3. Prepare most concentrated food-grade sample (“df 1”)
		1. Turmeric: To 9.5 mL BSC-GM, add 500 µL of filtered turmeric solution
		2. Carrot: To 9.3 mL BSC-GM, add 735 µL of filtered carrot juice
		3. V8 juice: To 9.6 mL BSC-GM, add 575 µL of filtered V8 juice
	4. Prepare serial dilutions with a dilution factor of 10 for each dilution
		1. For lab-grade solutions, to 4x 15 mL conical tubes, add 2.7 mL of BSC-GM with 0.1% of appropriate solvent. Label the tubes with 1,000 ng/mL, 100 ng/mL, 10 ng/mL, and 1 ng/mL
		2. For food-grade solutions, repeat as above but with just BSC-GM. Label tubes with dilution factor: df 10, df 100, df 1000, and df 10,000.
		3. To the first tube lab-grade solution tube (1,000 ng/mL), add 300 µL of the 10,000 ng/mL lab-grade carotenoid solution. Mix by pipetting up and down.
		4. Repeat the serial dilutions three times – 100 ng/mL, 10 ng/mL, 1 ng/mL – by adding 300 µL of the previous solution to the appropriate tube. Be sure to mix by pipetting thoroughly.
		5. Repeat the previous two steps but starting with the df1 food-grade solution. You will thus prepare df10, df100, df1000, and df10000 food-grade solutions.
3. With all your media prepared, add 200 µL media to your plates in triplicate, as in the layout below.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| **A** |  | BSC-GM | Food-grade df1 | Food-grade df10 | Food-grade df100 | Food-grade df1000 | Food-grade df10000 |  |
| **B** |  | BSC-GM | Food-grade df1 | Food-grade df10 | Food-grade df100 | Food-grade df1000 | Food-grade df10000 |  |
| **C** |  | BSC-GM | Food-grade df1 | Food-grade df10 | Food-grade df100 | Food-grade df1000 | Food-grade df10000 |  |
| **D** |  | BSC-GM with 0.1% solvent | Lab-grade 10000 ng/mL | Lab-grade 1000 ng/mL | Lab-grade 100 ng/mL | Lab-grade 10 ng/mL | Lab-grade 1 ng/mL |  |
| **E** |  | BSC-GM with 0.1% solvent | Lab-grade 10000 ng/mL | Lab-grade 1000 ng/mL | Lab-grade 100 ng/mL | Lab-grade 10 ng/mL | Lab-grade 1 ng/mL |  |
| **F** |  | BSC-GM with 0.1% solvent | Lab-grade 10000 ng/mL | Lab-grade 1000 ng/mL | Lab-grade 100 ng/mL | Lab-grade 10 ng/mL | Lab-grade 1 ng/mL |  |

1. To each well in the plate with media, add 10 µL of 100,000 cell/mL cell suspension to seed 1,000 cells per well
2. Place 48-well plate in incubator.
3. Feed with 200 µL of the appropriate media on Sunday and Tuesday.

References

1. Stout, A. J., Mirliani, A. B., Soule-albridge, E. L., Cohen, J. M. & Kaplan, D.L. Engineering carotenoid production in mammalian cells for nutritionally enhanced cell-cultured foods. *Metab. Eng.* **62**, 126–137 (2020).

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 |