Week 11: Cell Viability of Antioxidant Treated Muscle Cells

BME-0174

April 11th, 2024

General Notes

* + PrestoBlue is **light sensitive** so make sure to keep protected from light whenever possible
	+ PrestoBlue measure cell metabolism as a proxy for cell number (and general cell health)

Materials

* Antioxidant treated BSCs
* PrestoBlue reagent
* 96-well plate
* Plate reader
* BSC GM
* 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
* Conical Tubes
* T175 Flasks
* BSC cell suspension

Method

Viability Test

1. Add 20 µL PrestoBlue to each well
2. Add controls: to 3 empty wells, add 200 µL BSC-GM and 20 µL PrestoBlue to each well (this will be your “blank” reading)
3. Tilt plate to ensure PrestoBlue reagent is spread across well
4. Cover plate in foil and return to incubator
5. Incubate ~1 hour
6. Transfer 150 µL media + PrestoBlue from each experimental well into a 96 well plate (note which sample is in which well, see schematic below for reference)
7. Read plate at 560/590 nm
8. Analyze results:
	1. Subtract control/blank readings from each sample (average the blank readings and subtract this number from each experimental sample’s reading)
	2. Create a bar chart comparing each treatment
	3. Note any differences in cell viability with each treatment



Media Prep

1. Set aside 80 ml of BSC GM (this is the control group)
2. Prepare 80 ml of growth media with your chosen concentration of carotenoid (pure extract)
3. Prepare 80 ml of growth media with your chosen concentration of carotenoid (from juice)

Cell Seeding

1. Take a tube of cell suspension and divide evenly into 3x15 ml conical tubes
	1. BE SURE to evenly mix/homogenize the tubes prior to dividing them amongst the tubes
2. Label the tubes according to the 3 media your prepared
3. Centrifuge your tubes at 300 x g for 5 mins
4. Remove the supernatant from the spun down cell suspension
5. Add 5 ml of each media into its appropriate tube with cells and mix/homogenize the solution well
	1. Ensure that the solution is homogenous/single cell suspension/free of cell clumps
6. Add remaining 20 mL of each media to separate T175 flasks
	1. Label with the sample group, your group #, the class # and section, and the date
7. Add each 5 ml of cells + carotenoid media/normal media into each appropriate flask.
8. Transfer the flasks to 37C 5% CO2.
9. Feed on Sun and Tues.

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 |

References

1. https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0018371-PrestoBlueHS-CellViabilityReagent-PI.pdf