## TBARS Assay

**Date: April 25, 2023**

## Objectives, Hypotheses

We will “cook” and test our harvested cell pellets for malondialdehyde (MDA), a byproduct of lipid oxidation. This will help us verify or disprove our hypothesis that antioxidant treatment can reduce lipid oxidation in cultured meat.

## Materials Required

* Water
* TCA assay reagent
* Color Reagent
* MDA stock (125uM)
* 96-well plates
* Ice

## Protocol/Procedure

Sample cooking

1. Place pellet tubes in a 100C heat block for 10 minutes. Retrieve samples from the heat block.

Standard preparation

1. Prepare your standard curve:

|  |  |  |  |
| --- | --- | --- | --- |
| Tube | MDA (uL) | Water (uL) | MDA concentration (uM) |
| A | 0 | 500 | 0 |
| B | 20 | 480 | 5 |
| C | 40 | 460 | 10 |
| D | 100 | 400 | 25 |
| E | 200 | 300 | 50 |

Sample preparation

1. Add 200 uL water to each cell pellet
2. Lyse cells using freeze-thaw method.
   1. Place tube in –80C for 5 minutes
   2. Completely thaw
   3. Repeat for 3 cycles

Assay setup

1. Add 100 uL of sample or standard to appropriately labeled 1.5 mL Eppendorf tube (11 tubes total: 5 standards, 6 samples)
2. Add 100 uL of TCA assay reagent and pipette to mix
3. Add 800 uL of Color reagent to tubes
4. Cap vials and place on 100C heat block for 1 hr
5. After 1 hour, remove vials and place on ice for 10 minutes
6. Set microcentrifuge to 4C
7. After 10 minutes, centrifuge vials for 10 minutes at 1,600 x g and 4C
   1. Vials are stable at RT for 30 minutes
8. Load 200 uL (in triplicate) from each vial into a clear 96-well plate. Avoid bubbles and keep track of where in the plate you have placed which standards / samples.
9. Read absorbance at 535 nm

Assay analysis

1. Prepare a scatter plot of absorbance/MDA control (uM) and add a linear best-fit line
2. Using the y-intercept and slope of the best-fit line, calculate MDA uM from each of your samples, average between replicates and compare between conditions

## Data/Observations/Conclusions