Week 8 Experiment

TX-100 toxicity, assessed by Alamar Blue

Pointers for today

- Make your TX100 dilutions directly into the wells of your plate.
- Once you use your D10 Media at the bench, remember to label it with “Non Sterile”
- You can use a multichannel, but this often leads to inaccurate measurements.

TX-100 Toxicity or Permeability Assessed by Alamar Blue

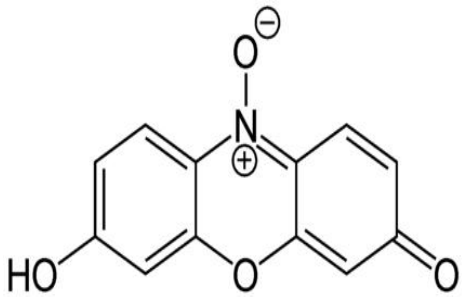
0	0	0.025%	0.05%	0.1%	0.25%	0.5%	1%	2.5%	5%	10%	0 *No Cells

Remove media from the 96 well plate, using a multichannel pipette. Take extra care to not disturb the cell monolayer. Add the calculated volume of D10 Media to each well with a multichannel pipette. You may use the same tips.

To the media, add the needed volume of 10% TX-100 with a single tip pipette.
Then incubate at your bench for 15 minutes.

Remove the TX-100 treatment. Add 100µl of fresh D10 and then 20µl of Alamar Blue. Incubate at 37˚C for >1.5 hrs

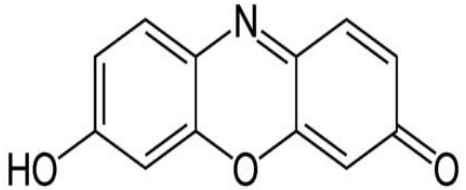
Alamar Blue:Redox



Resazurin

NADH / H^+

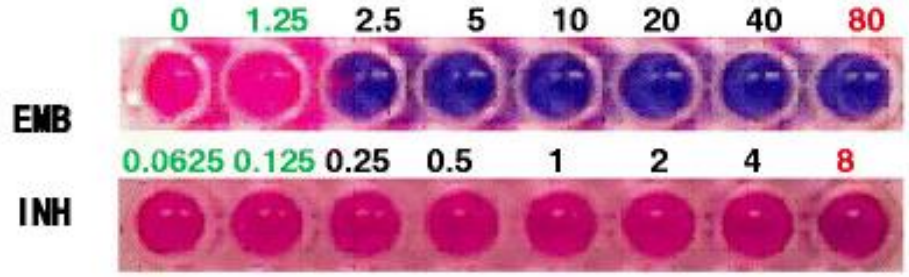
NAD^+, H_2O



Resorufin

Reduction: Gain of electrons
Oxidation : Loss of electrons

Resazurin: Blue and weakly fluorescent
Resofurin: Pink and fluorescent at 590nm



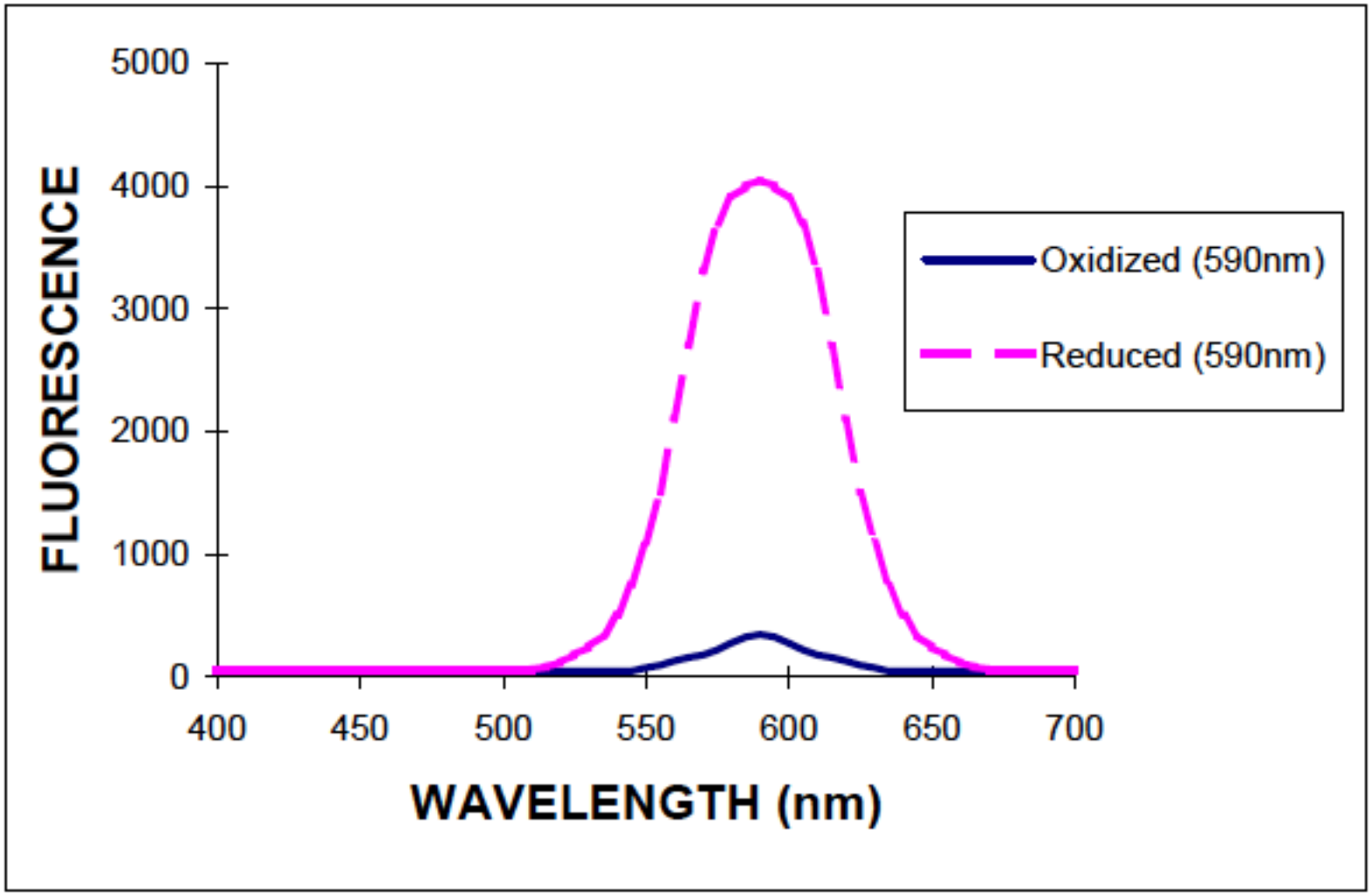
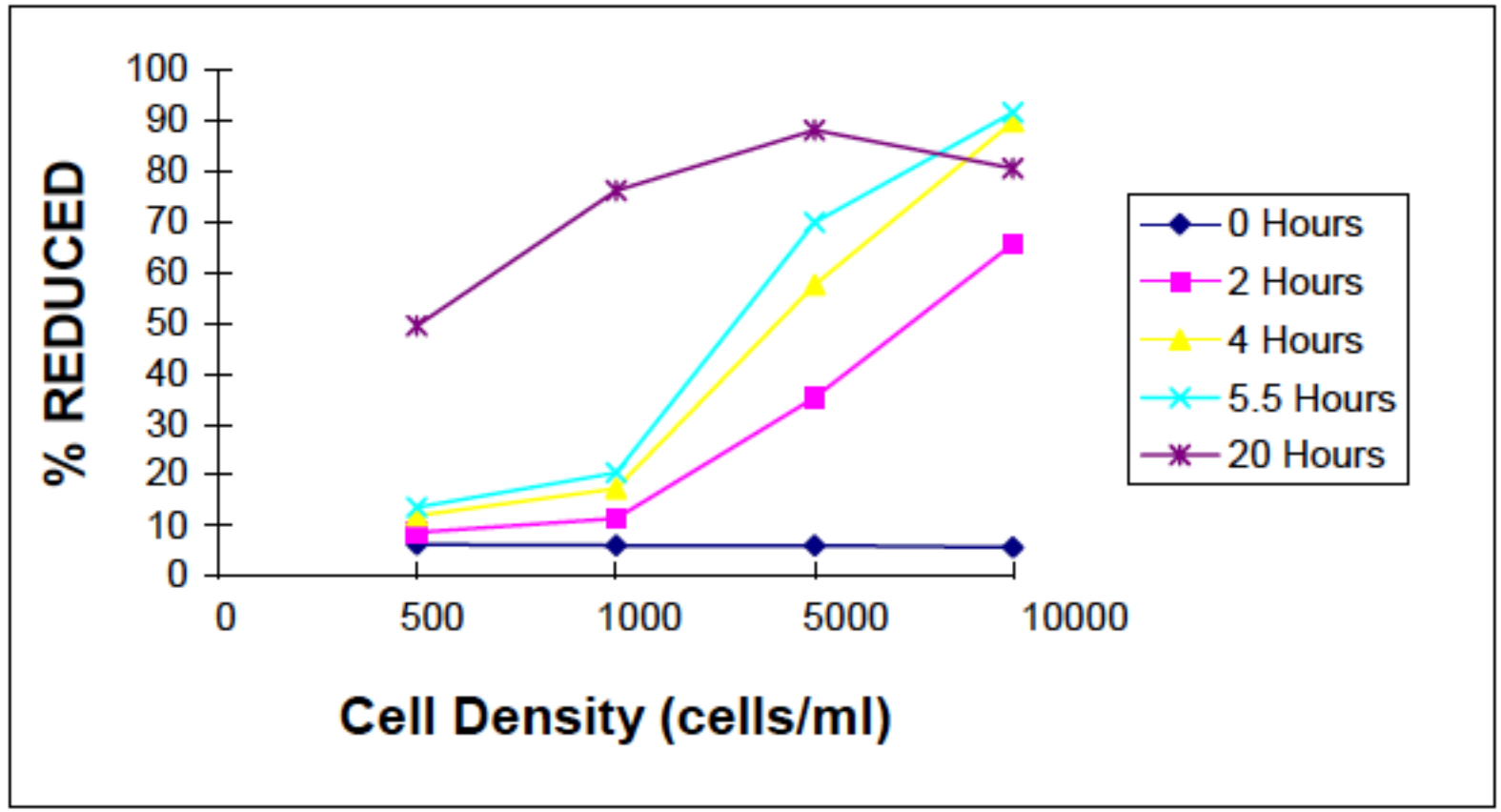


Figure 1a. alamarBlue® Fluorescence Emission Spectra


% REDUCED
100
90
80
70
60
50
40
30
20
10
0
0
500
1000
5000
10000
Cell Density (cells/ml)
0 Hours
2 Hours
4 Hours
5.5 Hours
20 Hours