CBEC "Cell Block" MTT Assay
Created by Lori Hensley and the CBEC

Before you start:

- Warm MTT reagent + media (49 mL plain media + 1 mL MTT reagent)
  - Plain media is media without the FBS, P/S, and L-glut.

MTT Assay

1. Look at your cells under the microscope. Note how they look in each treatment group.
2. First vacuum off the media.
   - Put vacuum pipet on vacuum tube. Make sure to only touch the very end of the pipet.
   - Carefully tilt plate (with cells) and vacuum off the media from the front of the well.
     - Remember the cells are stuck on the bottom of the well. Keep this in mind as you go through the procedure.
   - When the media is completely vacuumed, carefully remove the pipet from the vacuum tube making sure to let the last few drops make it down the vacuum tube.
   - Throw away the vacuum pipet in the cardboard bin to the left of the hood marked "glass."
3. Add MTT reagent + Media to each well
   - For a 24 well plate: add 0.5 mL/well
   - For a 96 well plate: add 100µL/well

   *You can take up 6 mL and carefully let out only 0.5 mL at a time into each well. Gently touch pipet to the side of the well with the pipet tip and release liquid in order to make sure you don't knock off the cells from the bottom of the flask.

Belly Dancer

4. Incubate for 1-2 hours
5. Vacuum off media 3 at a time
6. Add DMSO to all wells + 3 wells for blanks
   - For a 24 well plate: add 0.5 mL/well
   - For a 96 well plate: add 100µL/well

   *Use same technique as above.
7. Put plate on the belly dancer for 10-20 minutes
   - In room 119 beside the back fridge
8. Take a picture of your plate.
9. Read in the plate reader according to the *Plate Reader Protocol*.
   - Use the predefined method "HensleyMTT-24 well" in the "Methods" folder.
10. Save the excel file in My Documents→ Cell Biology Fall 2015→ Your group folder.
11. Email the excel file to yourself.
    - You must use internet explorer on this computer.
    - Chrome will not attach the file.
12. Graph results.
    - May occur at a later date.