MTT Assay

Day 1: Change media. For confluent cell lines, reduce cell number by half.

Day 2: Plate cells in 96 well plates

1. trypsinize and pellet cells
2. resuspend pellet in 3 or 5 ml of 10% serum media depending on pellet size
3. syringe 5-10 times through 18 gauge needle to get single cell suspension, vortex
4. add 10 µl cell suspension to 90 µl trypan blue, count with hemacytometer
   # of cells/ml= # of cells/4 x dilution factor x 10^4
   dilution factor=10
5. dilute cells to 1 x 10^5/ml in 10% serum media, vortex
6. count cells again (no trypan blue- undiluted)
7. dilute cells to desired concentration, vortex
   for 4,000 cells/well, dilute cells to $4x10^4$/ml
8. plate 100 µl per well (10 rows of 6, leave empty wells around the outside)

always vortex before counting or diluting to assure even cell suspension

Day 3: Add drug

1. plan dilution scheme based on desired concentration of drug
2. dilute drug in serum-free media, **vortex in between each serial dilution**
3. add 100 µl per well
4. add 200 µl sterile water per well to each empty well

Day 8: Add MTT

1. make solution of 2 mg MTT/ml serum free media (heated to 37° C)- need 3.3 ml/plate
2. sterilize MTT solution with syringe filter
3. add 50 µl per well, including a blank row
4. leave in incubator for 4 hours
5. suck off liquid using aspirator in the lab- make sure not to suck off purple solid in the bottom of the wells
6. add 100 µl solubilizing solution, pipet up and down to mix
   for 400 µl solubilizing solution: 92 µl DI water, add 8 µl HCl to the water, add that to 300 µl propanol
7. read plate at 490 with reference wavelength of 650, automix once before reading

10 mL = 2.3 mL H_2O
100 µl HCl 10N
7.5 mL iso

20 mL 4.6
200 10N
15 mL