Cell proliferation assay in 96 well plate

1. Trypsinize cells :
* Aspirate growth media
* Add trypsin- 2 ml per T75 flask
* Transfer to the incubator for 2 min
* Look under the microscope to check if the cells are detached, if not incubate for additional time as needed
* Add 5ml growth media and pipette up and down to make sure cells detached and are not in clumps, but singles
1. Count cells :
* Mix cell by pipetting and take 200ul to a new eppendorf for counting
* In a new eppendorf ,mix 15ul of the cells and 15ul of trypan blue, and load 10ul to the counting slide, in a duplicate (A and B)
* Count cells using the countessII FL
1. Make calculation of cells dilutions in growth media to reach the appropriate number of cells in each well, for example- 5000 cells per well in a 96 well plate, use the file below to calculate :

[poliferation calculation template.xlsx](file:///C%3A%5CUsers%5Cmoshee%5CAppData%5CLocal%5CMicrosoft%5CWindows%5CTemporary%20Internet%20Files%5CContent.Outlook%5CBBXAI2XO%5Cpoliferation%20calculation%20template.xlsx)

1. Seed cells in 100ul final volume in each well in a 96 well plate, arrange the plate according to treatments needed, and label the cover accordingly.
2. Shake
3. Look under microscope to verify seeding (cells should be separated , low density and evenly distributed in the wells)
4. Treat cells with different compounds as indicated in excel file you prepared
5. Transfer the cells to the incubator for the appropriate time. Typically, a 5 day experiment is sufficient.
6. At the end of the experiment, cells are fixed, stained with crystal violet and the intensity of the staining is calculated (indicates number of cells):

Fixation:

* + Remove the media from each well
	+ Fix the cells using 10% TCA (Trichloroacetic acid) by adding 100ul 1hr at 4 0C.
	+ Aspirate and rinse with tap water X5 times
	+ Air dry the plates
	+ Add 50ul of crystal violet solution to the plates, 10 min, RT
	+ Rinse with tap water X5, and dry. Make sure they are completely dry
	+ Dissolve the dye using 50ul 10% acetic acid (10 ml acetic acid+90ml ddw)
	+ Read absorbance at wavelength 570nm
	+ Extract data and compare the number of cells in each treatment versus the control (typically DMSO)
* **Crystal violet:**

Make 1 L of crystal violet solution:

0.1% w/v of crystal violet (1 gr /L) in ddw

* **TCA:**

The final concentration of TCA to be used is 0.6M

Take 10ml of TCA (6M) and 90ml of ddw to final 100ml, 10% TCA.