MTT Cell Assay Protocol

Day 1: Set up

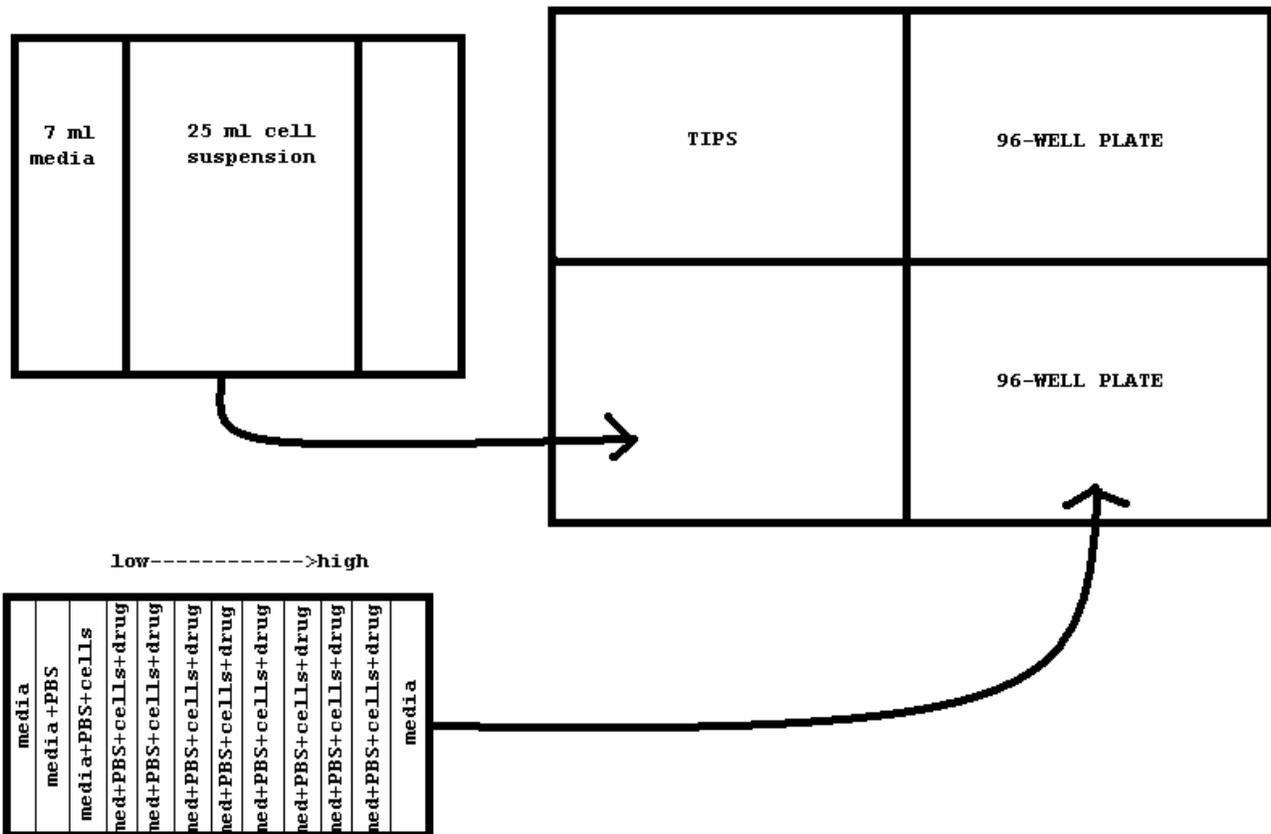Cell Dilution
1. Harvest cells, either by centrifugation (if suspension) or by trypsin
1. Stain 90ul cell suspension with 10ul of Trypan blue.
2. Determine whether the cells have greater than 90% viability.
3. Determine a cell count
4. resuspend cells at the density needed for the MTT assay:
    a. Density should be determined by a density experiment. In general for leukemic cells, a density of 0.5-1.0x10^5 cells/ml works. For primary leukemic samples (which don't divide, plate at 1x10^6 cells/ml. For solid tumors, the density varies from 1x10^4 to 1.5x10^5, so you will need to determine the appropriate density for each cell line.
    b. For two plates of cells, you will need a total of 25ml of cells suspended in media.
5. Calculate the proper number of cells needed for assay:

    ie. 23/2 (23 alive cells/ 2 dead); cells needed: 1.5x10^5

    (1.5x20^5cells)(25ml)
    -------------------------- = 10.8 ml cell suspension to be diluted to 25ml with media
      2.3x10^5cells

Machine and Plate Set-up

7 ml media	25 ml cell suspension	

TIPS	96-WELL PLATE
	96-WELL PLATE

low------------>high

| media	media+PBS	media+PBS+cells	med+PBS+cells+drug	med+PBS+cells+drug	med+PBS+cells+drug	med+PBS+cells+drug	med+PBS+cells+drug	med+PBS+cells+drug	med+PBS+cells+drug	med+PBS+cells+drug	media

** Label your plates with tumor type, passage, drug, date, and name on cover. Mark plate along the side so that you know which top belongs to which plate.

1. Turn on machine/ computer (button by left foot)
2. Run method
3. Choose program CellPZA. Press "enter" 9 times to begin.
4. Incubate your plates in 37C, 5% CO_2 overnight. (suspension cells can be plated the same day and incubated for several hours, but it's best to do all the plating the day before drug is added to decrease variability.

Day 2: Adding the Drug

1. Dry modules for drug dilutions. For each drug, you will need four-1/8 modules and one quarter-module for PBS
2. Determine amount of PBS and drug needed using the MTT single drug assay sheet. Serial dilutions of 1:10 work well for initial experiments, 1:3 or 1:4 work well when you have a ball-park idea of the IC50 for that drug in your cells.
3. Set up wells for drug addition. Add PBS into each well.

Lowest   Highest
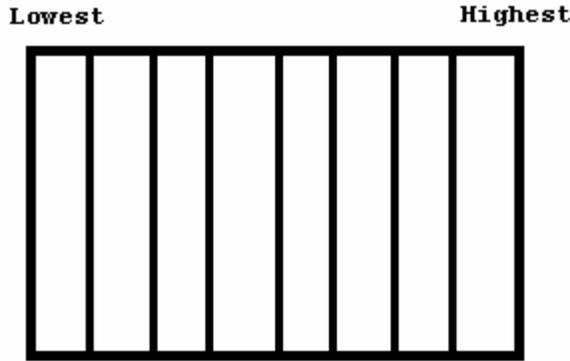
4. Make drug dilutions starting with highest concentration and working down. Make sure to mix each module thoroughly before making the next dilution
5. Do a serial dilution of drug stock.

   Ex:

      Drug Stock Conc. 1uM or 100nM
      Dilute to: 50nM
                                25nM
                                12.5nM
                                etc…

   Put an additional 3ml PBS in the first well and add 6ul of 1uM drug stock.
   Mix and remove 3ml of the drug dilution to the next well, continuing the process from right to left until you end up with 6ml of diluted drug in the last well.
   **note: if drug is light sensitive be sure to work in the dark

6. run program: PBSA2-H3 to add 15ul PBS to columns 2 and 3
7. To add drug: Run program: ADDPZA2 for 2 plates/ ADDPZA for 1 plate
   ** these programs add 15ul of drug from the 8 wells from columns 8-11
8. After drug has been added, place the plates on the shaker for 3-5 min. on Setting 5.
9. Incubate plates for 72 hrs. @ 37C. cover with foil if light sensitive.

Day 3: Adding MTT and Reading Plates

Adding MTT
1. Fill a quarter module with a 2ml reservoir + 2ml/plate of MTT (ie. For 6 plates, need 14ml MTT)
2. Place plates in proper position (on the left side of an empty cartridge)
3. Run prgrm: MTT-15ul
4. Shake the plates for ~5min @ setting 6
5. Incubate 4 hrs.

**note: to add MTT to only 1 plate you should manually stop the machine by pressing " \S " to stop then " \A " to abort the program. Next place the MTT filled cartridge up to the tips and press "enter" to discard the remaining solution in tips. Press "esc" to get back to the main menu.

Preparing Plates for Reading

1. Spin the plates down for 5 min. @1000rpm (for suspension cells)
2. Place half-module on the left side of an empty cartridge and select program: WASH150 (this program takes out 150ul media out of each well)
   **note: dump filled modules into waste bottle as it fills
3. Meanwhile prepare a half-module filled 4/5 to the top with DMSO (you will need this amount of DMSO for every two plates, refill as you continue with more than 2 plates)
4. When each plate has undergone WASH150 program, replace the module containing the supernatant with the module filled with DMSO and select program: DMSO150 (adds 150ul DMSO to each well)

Reading Plates

1. Turn on plate reader first, then the computer and printer
2. @ the prompt type: mlab/parcom
3. @ next prompt type: arcom30
4. Press enter @ first choice in Main Menu
5. Type in your filename: ie. "JC051205a" initials, date, plate identification (ie.a-f)
                        filter: 550
                        Press F1to collect data
6. For next sample, rename your file and press F1 to collect data
7. Press Escape, then F1 to show data
8. Select plate to be printed and press F9
   **note: to get back to main menu press "ESC"
9. To copy the file: ESC out of program and type "copy JC041205a.msw a:" IF you have multiple files with the same shortcut, you can use * as a wildcard.
10. run data through Excel program to calculate cytotoxicity.