NYU School of Medicine
Department of Microbiology

Ana Rodriguez, Ph.D.
Associate Professor
Ana.Rodriguez@nyumc.org

Cytotoxicity assay for HTS in 96 well plates with Alamar blue

- Final volume per well for cells+compound is 100 μl.
- Compounds are tested in duplicate.
- Final volume after adding Alamar Blue is 110 μl.
- The assay is performed FOR 4 DAYS ASSAY in DMEM **without Phenol red +** 2% FBS and 1% PSG to avoid interference of phenol red with the 590 nm absorbance reading.
- **Controls**
    1. cells alone
    2. dead cells: cells + Ionomycin 100 μM or other drug or detergent
    3. medium alone

Protocol:

- warm up medium
- Trypsinize NIH/3T3 or HepG2 cells as described in cell culture protocol.
- When cells are detached, harvest them in DMEM **without Phenol red +** 2% FBS and 1% PSG.
- Spin for 5 min at 1000 rpm.
- Throw medium.
- Resuspend in DMEM **without Phenol red** + 2% FBS and 1% PSG.
- Spin again 5 min at 1000 rpm.
- Resuspend and count.
- Dilute cells to $5x10^5$ cells/ml, transfer to sterile basin and plate 100 μL (50.000 cells) per well with multichannel pipette.
- Put back in incubator for 3 hours to allow cells to attach.
- Defreeze compounds.
- Vortex compounds.
- Add 2 μL of drug in the first row + 98uL of fresh media. Pipette up and down carefully to mix and transfer 100uL to the next row in order to make serial dilutions (2 fold dilution)
- Incubate for 4 days and add 10 μL Alamar blue

November 1, 2016