

**CYTOTOXICITY ASSAY
FOR TARGETED TOXINS *IN VITRO***

DAY 1:

1. Transfer 100 μ l of media into the wells around the edge of a 96-well plate. These wells simply offer some protection for the experimental wells.
2. Determine the number of cells needed for the planned number of plates. Cells are plated in the center 60 wells in 90 μ l of media per well. Cells are usually plated at 500 to 5000 cells per well (The slower your cells grown, the more cells per well you want to use).
3. Allow cells to acclimate overnight.



DAY 2:

4. Add the test samples in 10 μ l volumes. The initial concentration of targeted toxin is 10-100 nM. Dilute 1:10 across the plate to establish a curve. Generally the highest concentration of targeted toxin is 10 nM.
5. The first and last experimental columns (2 and 11 in most plates) of cells are controls, only medium is added to these wells.
6. Incubate 72 hours.

DAY 5 (72 hours after adding test samples):

7. 1.5 ml of MTS and 75 μ l of PMS are needed per plate. (These reagents can be purchased from Promega, part of the CellTiter 96 AQueous Nonradioactive Cell Proliferation Assay Kit, catalog #G5421).
8. Add 20 μ l of PMS/MTS to each sample and control well. Add 20 μ l to well A1. This is your blank.
9. Develop 20 minutes to several hours in the incubator. The incubation time will depend on cell number and type. (The first time you work with a particular cell line you should check every 20 minutes to see if there is a significant color change. There will be a noticeable difference in color between the control cells and the blank.)
10. Read the plate in the spectrophotometer at 490 nm. Try for an absorbance value in the 0.3-0.8 range. If the absorbance is not high enough, the plates can be returned to the incubator.
11. The absorbance of the sample wells is compared to the control wells to establish a curve. The ED₅₀ of the targeted toxin can be derived from the curve.

