**Protocol graciously provided by: Dr. Mariano Viapiano, Viapiano Lab protocols (© 2006-2014)**

## I. Measurement of cell viability on nanofibers

1. Cell viability can be measured directly on 96 well nanofiber plates using the soluble formazan reagent CellTiter from Promega (single solution and comes already prepared). It is essentially identical to measuring cell viability in a plastic multiwell plate.
2. Plate dissociated cells as indicated in protocols G-H (*Staining and seeding dissociated adherent cells on nanofiber plates*, and *Preparation and culture of cell spheroids on nanofibers* respectively), in presence of test compounds or vehicle.
3. Recommended starting cell numbers are 4,000 for adherent cells and 5,000-10,000 for dissociated glioblastoma stem cells (final volume = 100 µl/well).
4. Add 20 µl CellTiter per 100 µl medium and incubate at 37 ˚C for 1-4h until you can see color development. Usually adherent cells (U87, U251, etc) will take about 1h for this and stem cells 2 to 4h.
5. Shake the nanofiber plate gently and transfer 100 µl of medium to a clean microtiter plate
6. Measure absorbance at 490 or 495 nm (background correction at 620-630 nm) using a plate reader.
7. The reaction is based on the ability of viable cells to reduce a soluble tetrazolium compound in the CellTiter reagent, yielding a yellow-brown product that can be quantified by absorbance.