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G) Measurement of cell viability on nanofibers

- Cell viability can be measured directly on 96well 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.
- Plate dissociated cells as indicated in sections E-F, in presence of test compounds or vehicle.
- 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).
- 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.
- Shake the nanofiber plate gently and transfer 100 µl of medium to a clean microtiter plate
- Measure absorbance at 490 or 495nm (background correction at 620-630nm) using a plate reader.
- 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.

H) Measurement of cell toxicity on nanofibers

- Cell toxicity can be also measured directly on 96well nanofiber plates using an LDH quantification kit (from Clontech, Roche Applied Science, or Sigma-Aldrich). The procedure is also identical to measuring cell viability in a plastic multiwell plate.
- The kit used in the lab is the LDH detection kit from Clontech. Two reagents of this kit (Catalyst and Dye solution) can be prepared in advance, aliquoted, and stored at 4 °C for several weeks.
- Plate dissociated cells as indicated in sections E-F, in presence of test compounds or vehicle.
- Recommended starting cell numbers are 10,000 for adherent cells and 20,000 for dissociated glioblastoma stem cells (final volume = 150 µl/well).
- If adherent cells require serum, reduce the serum content in the medium to 1% and make sure that it is heat-inactivated. Serum contains LDH that will yield false positives.
- After one day in culture, add 50 µl of culture medium to the wells containing the test compounds or vehicle. Incubate for the time required to test for toxic effects (usually 2-24h). As a positive control (complete cell lysis), treat the cells with 2% Triton X100 in the culture medium.
- Shortly before the end of the experiment, prepare enough LDH reaction mixture (1 part catalyst:45 parts dye) for the number of samples to be tested (100 µl of mixture per sample).
- Using a centrifuge with multiwell adapter, centrifuge the plate at 250 g x 10min.
- Collect 100 µl of supernatant from the wells and transfer to a 96well microtiter plate.
- Add 100 µl of fresh LDH reaction mixture and incubate at room temp in the dark for 30min.
- Measure absorbance at 490 or 495nm (background correction at 620-630nm) using a plate reader.
- If the values are too low, incubate the plate at room temp in the dark for another 30min and repeat the measurement.
- The reaction is based on the release of the enzyme LDH from damaged cells to the culture medium. The Clontech reagent uses a substrate that is reduced by LDH, yielding a color product that allows quantification of the enzyme.