MTT Assay of Cell Viability Protocol
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Supplies
- Cultured cells in a 96 well plate with at least three empty wells. At least three wells should be absent of media for 12 hrs+ for control condition. Grown in sub-confluent monolayers. Have 150 µL of media per a well at time of experiment.
- MTT: Thiazolyl Blue Tetrazolium Bromide
- Shaker plate

Procedure
1. Make up an MTT solution at a concentration of 0.5 mg/mL in media at a volume equivalent to 150 µL per a sample, multiply amount by 1.2 to account for 20% error.
2. After aspirating off culture media, add 150 uL of MTT solution to each well of the cell plate, including 3 blank wells. Gently shake the plate to mix the solution. Ensure solution does not splash into other wells.
3. Place lid or 96-well plate sticker over the plate. Wrap with aluminum foil. Spray down with 70% ethanol and place in cell incubator (37° C, 5% CO2) for 3-4 hours (some cells require more or less time).
4. After incubation, aspirate off the media from the wells using a Pasteur pipet and the vacuum in the laminar flow hood.
5. Resuspend the formazan (MTT metabolic product) in 100 µL isopropanol.
6. Place plate on a shaking plate at a medium speed for at least five minutes, until the crystals have dissolved. Ensure the solution does not splash into other wells.
7. Read in a plate reader at 560 nm. Subtract background at 670 nm.
8. The blank wells will also serve as background.

Troubleshooting-
- If you are getting too many crystals and they are not dissolving, then you have too many viable cells. You need to seed sparser or allow less growth time, or expose the cells to MTT solution for only 1-2 hours.
- Optimally, you should aim to replicate all of your experimental samples to run the MTT assay at a single time. If you have to run the MTT several different times to get a proper n, then use a consistent incubator and crystal dissolution time in steps 3 and 6. This is the only way the data can be validly compared, assuming all other conditions are kept constant.