## **MTS Cell Proliferation Assay**

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**Purpose**: The CellTiter 96 Aqueous One Solution Assay (Promega) is a colorimetric method for determining the number of viable cells in proliferation or cytotoxicity assays. The One Solution contains MTS compound and an electron coupling reagent PES. The MTS compound is bioreduced by cells into a colored formazan product that is soluble in tissue culture medium. The quantity of formazan product as measured by the amount of 490nm absorbance is directly proportional to the number of living cells in culture.

- 1. Plate cells in triplet with 96-well plate and 2 hours later infect the cells with the proper titer of virus.
- 2. 24 hours after infection, replace the medium with 100ul fresh medium, and add 20ul of CellTiter 96 AQueous One Solution (Promega, Cat.#: G3582). Set up a triplet "no-cell" control containing 100ul of culture medium and 20ul of One Solution at the same time. Return the plate to incubator for ½ to 4 hours (I incubated for one hour with BMP9/IDs-infected C2C12 cells).
- 3. Transfer 60ul medium of each well to an ELISA 96-well plate, and record absorbance at 490nm (450-540nm) with a 96-well plate reader. Subtract the average 490nm absorbance from the "no-cell" control from all other absorbance values yields the corrected absorbance. (Background absorbance from "no-cell" control is typically 0.2-0.3 absorbance units after 4 hours of incubation)