Assay Name: 3D multicellular tumor spheroid (MCTS) endpoint viability screening assay

Assay ID: Celigo_03_0007

Description: Measure the effects of a panel of drugs on the viability of U87MG Glioblastoma MCTS using calcein AM and Propidium Iodide fluorescent staining

Stains: Calcein AM and Propidium Iodide

Imaging channels: Green, Red, and Bright Field

Image analysis algorithm: Celigo software Tumorsphere 1 + 2 + Mask

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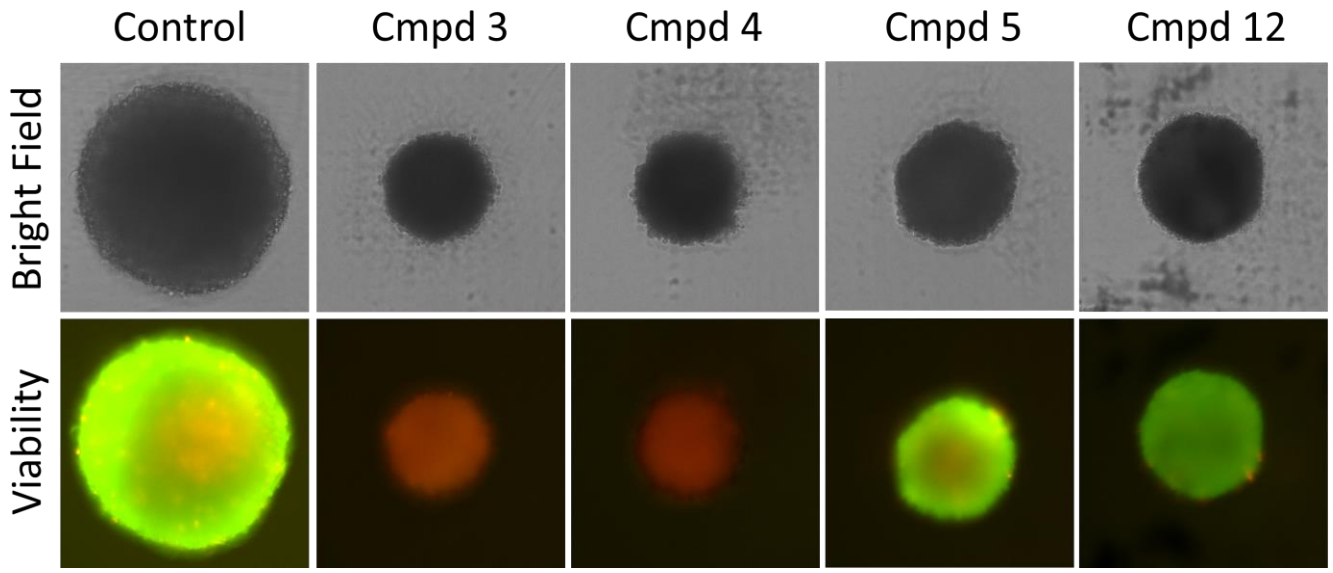
Methods:

1. Seed 500 U87MG cells/well in ULA 384-well plates
2. On day 4, add different diluted drug compounds at 2x and a vehicle control in media
3. On day 13, prepare and stain the MCTS with the calcein AM and PI
4. Incubate the plate at 37 °C and 5% CO2 for 60 min
5. Image and analyze on Celigo
6. Compare the spheroid viability for the tested drug compounds

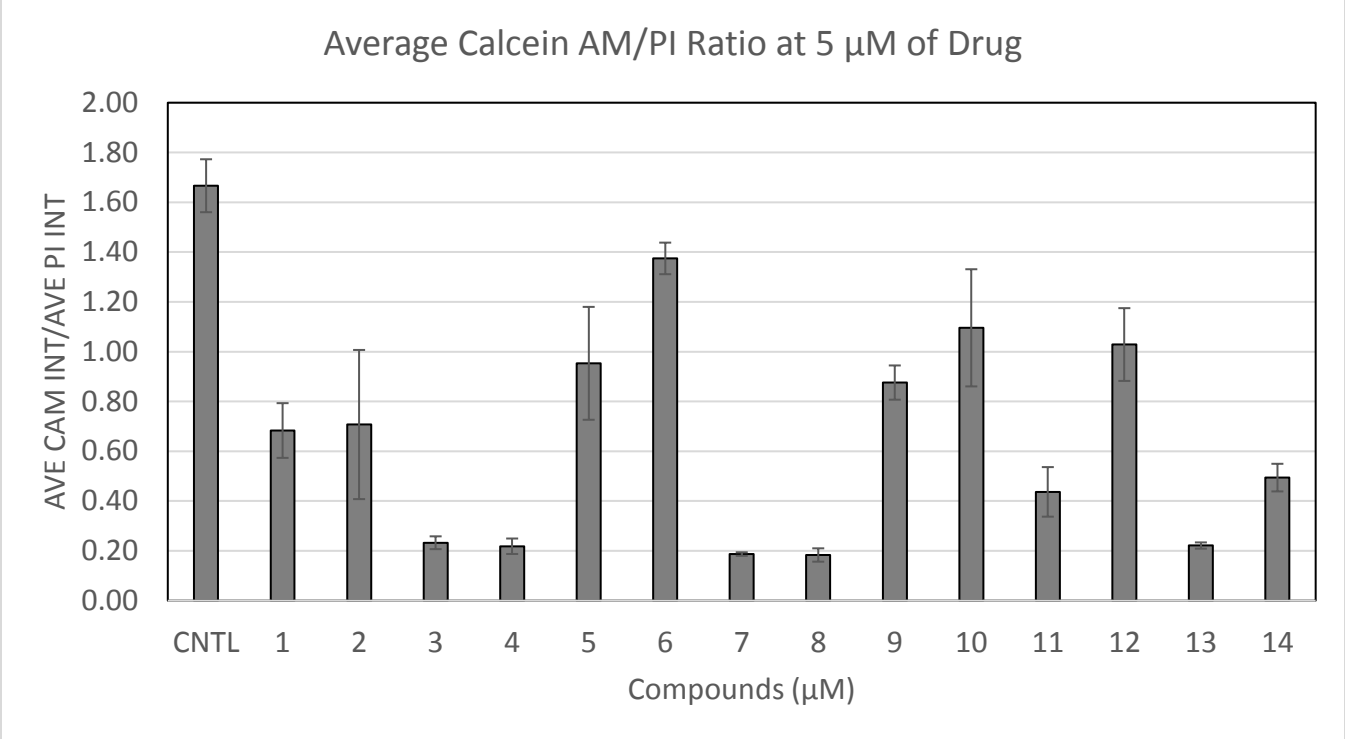
Results:

Celigo-captured bright field and fluorescent images showing calcein AM/PI staining of the U87MG MCTS



- The bright field images were used to identify the spheroids in the well
- The calcein AM and PI fluorescent intensities were measured from the images
- Calcein AM/PI intensity ratios were calculated to determine the viability

Endpoint measurement of 3D MCTS viability



- The plot above is showing the calcein AM/PI fluorescent intensity ratios, which indicates the viability of each MCTS treated with the different drug compounds
- Few drug compounds had moderate to high cytotoxic effects on the U87MG MCTS, while other drugs had no effects
- This method allows researchers to quickly identify the effects of the drug compounds on tumor spheroid viability in a high throughput manner