

Assay Name: 3D multicellular tumor spheroid endpoint apoptosis screening

Assay ID: Celigo_03_0005

Description: Measure the effects of a panel of drugs on the apoptosis of U87MG Glioblastoma MCTS using Caspase 3/7 and Hoechst fluorescent staining

Stains: Caspase 3/7 and Hoechst

Imaging channels: Green, Blue, and Bright Field

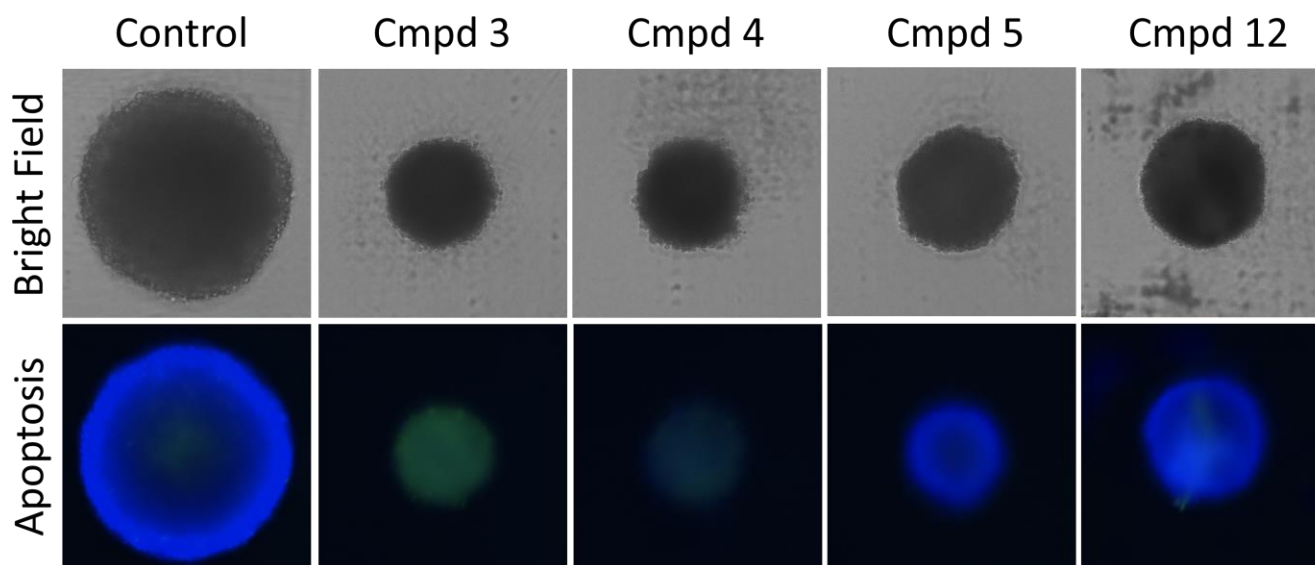
Image analysis algorithm: Celigo software Tumorsphere 1 + 2 + Mask

Methods:

1. Seed 500 U87MG cells/well in ULA 384-well plates
2. On day 4, add different serially diluted drug compounds at 2x and a vehicle control in media
3. On day 13, prepare and stain the MCTS with the Caspase 3/7 and Hoechst
4. Incubate the plate at 37 °C and 5% CO₂ for 60 min
5. Image and analyze on Celigo
6. Compare the spheroid apoptosis for each drug compound at each time point to characterize the tested compounds

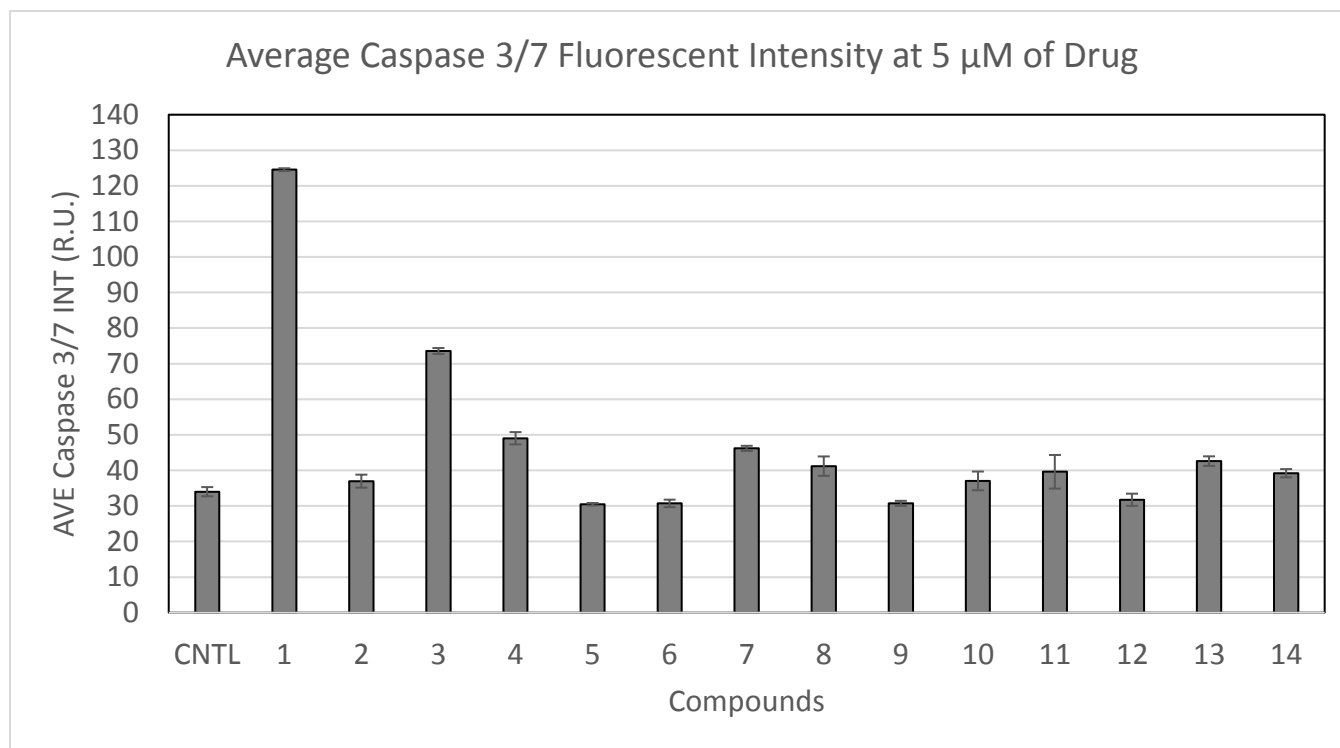
Results:

Celigo-captured bright field and fluorescent images showing Caspase 3/7 and Hoechst staining of the U87MG MCTS



- The bright field images were used to identify the spheroids in the well
- The caspase 3/7 fluorescent intensities were measured from the images

Endpoint measurement of 3D MCTS apoptosis



- The plot above shows the Caspase 3/7 fluorescent intensities, which indicate the apoptosis of each MCTS treated with the different drug compounds
- Only 2 drug compounds induced noticeable apoptosis on the U87MG MCTS, while other drugs had no effects