

## Assay Name: 3D MCTS Kinetic Propidium Iodide Assay

Assay ID: Celigo\_03\_0008

**Description:** Monitor the growth and signal intensity of dead cell stain, Propidium Iodide (PI), kinetically on U87MG Glioblastoma multicellular tumor spheroids (MCTS) for 10 days

**Stains:** Propidium Iodide

**Imaging channels:** Bright field, Red

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

### Methods:

1. Seed different cell numbers from 3,200 to 100 U87MG cells/well in ULA 96-well plate
2. Add PI and vehicle control in media to the plate. Spin the plate at 2,000 rpm for 10 min
3. Incubate the plate at 37 °C and 5% CO<sub>2</sub> until MCTS are formed
4. Image and analyze on Celigo for 10 days
5. Measure and graph the intensity of PI and diameter of MCTS for 10 days

### Results:

Figure 1. Bright field and red fluorescent images of U87MG MCTS with PI (red) over 10 days

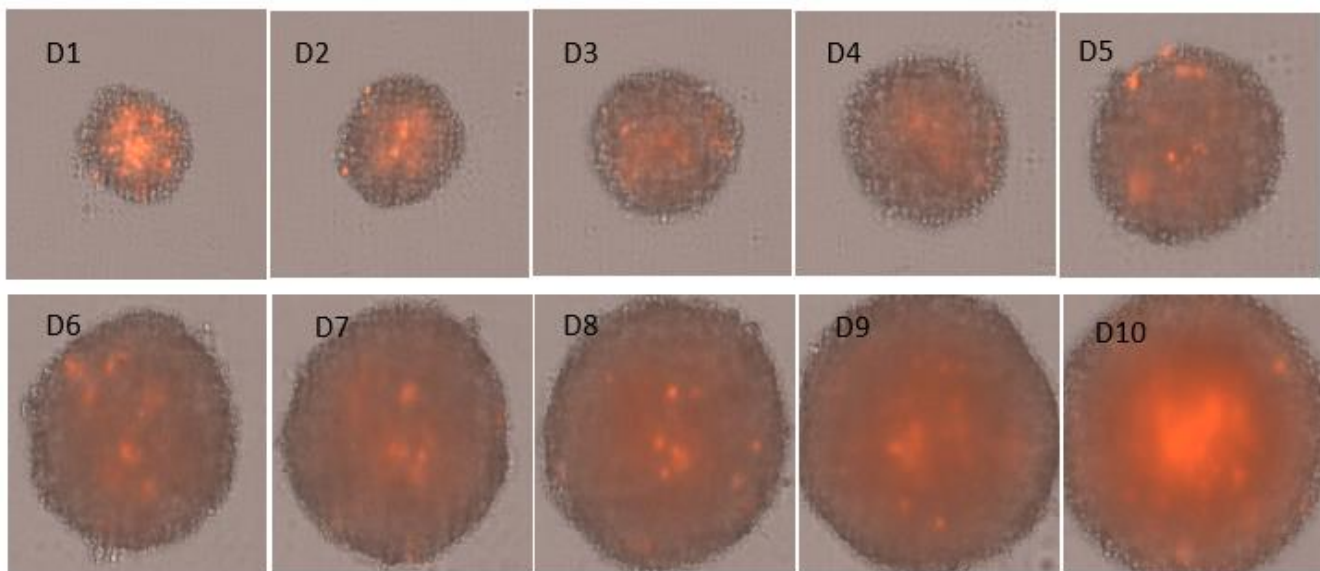
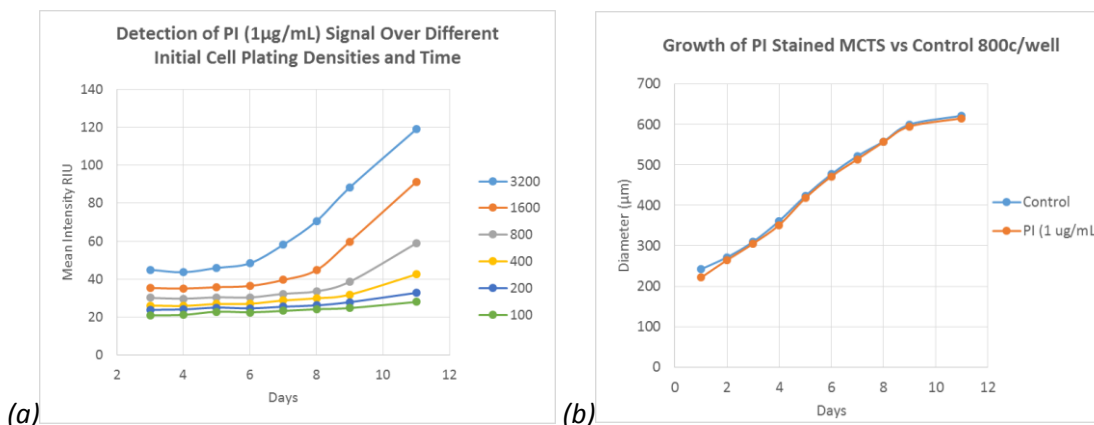


Figure 2. PI dead cell signal intensities and sphere diameter over 10 days

Line graph showing the trends of PI intensity for different cell concentration for 10 days (a). Line graph showing that there is no toxic effect of PI on MCTS diameter (b).



**Conclusion:**

- Using the Celigo image cytometer, images of U87MG MCTS were successfully captured and analyzed for growth and PI dead cell signal intensities
- Adding PI at the beginning of the experiment allowed for kinetic monitoring of cell death in the core of the MCTS
- As the sphere diameter begins to plateau, the signal of PI increases
- The entire 96-well plate was imaged in 2 minutes. The short scan time significantly increases the throughput during an experiment that has multiple plates