

Assay Name: Cell viability analysis using calcein AM, Hoechst and PI

Assay ID: Celigo_02_0002

Description: Analysis of cell viability on A549 cells using calcein AM, Propidium Iodide and Hoechst

Stains: calcein AM (green total live cells); Propidium Iodide (red dead cells); Hoechst (blue cell nuclei)

Imaging channels: Green, Red, Blue

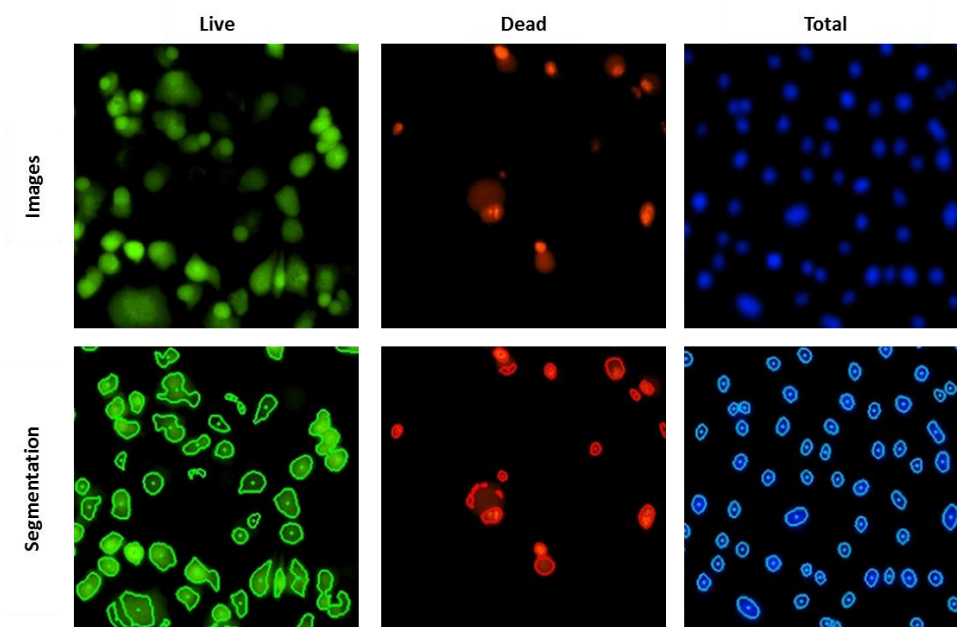
Image analysis algorithm: Celigo software Cell Viability (Live+ Dead + Total)

Methods:

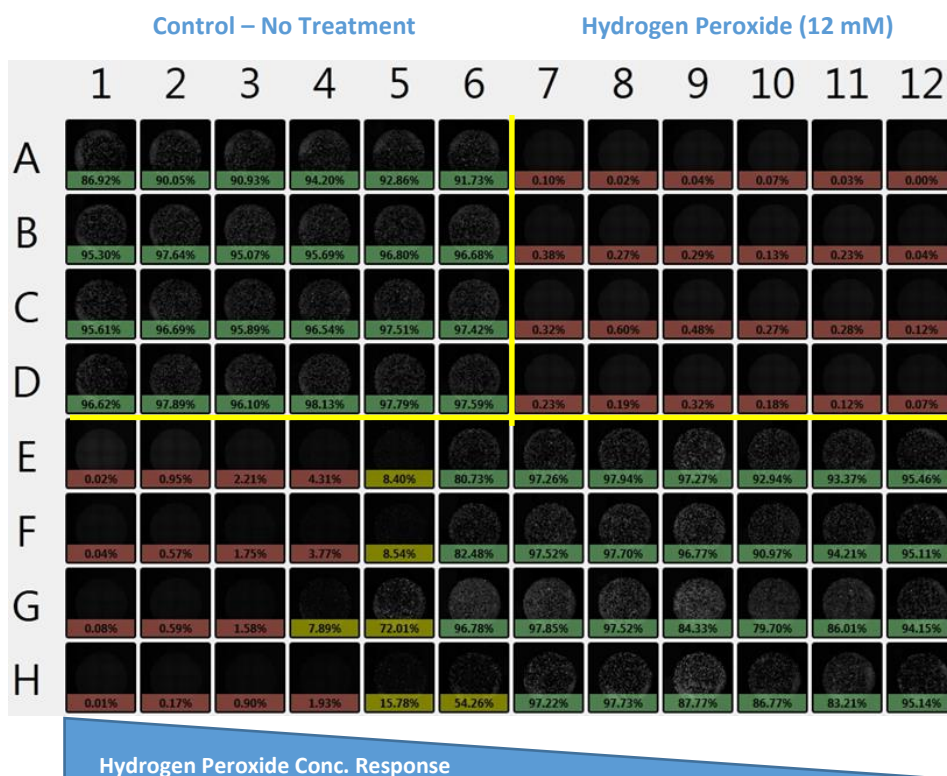
1. Seed cells in 96-well plate and incubate cells overnight
2. Add drug compound at 2x desired final concentration and incubate for desired time
3. Wash wells with media twice
4. Prepare and add calcein AM, Propidium Iodide and Hoechst 2X mixed dye solution
5. Incubate cells for 30 min
6. Acquire a whole-well image of an entire 96-well plate on Celigo ~ 15 minutes

Results:

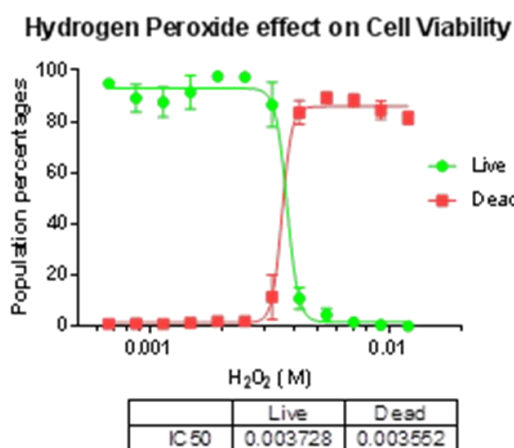
Viability measurement of A549 cells labeled with calcein AM, Propidium Iodide and Hoechst



- Calcein AM detects enzymatic activity in live cells, Propidium Iodide detects dead cells with compromised membranes and Hoechst is a marker for all nucleated cells.
- Identification and segmentation of live, dead and all cells are used to report the counts and percentages of live, dead and total numbers of cells.



- Whole plate readout with the heat map applied. Green: well containing a high % of live cells; Yellow: for wells with an intermediate level of live cells; Red: for a well containing a low % of live cells



- The plot above was generated by exporting the data into graph pad prism and graphing the percent of viability over the hydrogen peroxide concentration.