

Assay Name: Kinetic viability using PI

Assay ID: Celigo_02_0011



Table of Contents

Experiment: Kinetic Viability using Propidium Iodide	2
Celigo Setup.....	2
Assay Protocol and Plate Setup.....	2
Results	3
Drug-treated MDA-MB-231 and K562 cells showed an increase in PI-positive cells.....	3
Conclusion	4

Experiment: Kinetic viability using Propidium Iodide

Purpose	Perform kinetic viability assay on MDA-MB-231 and K562 cells
Current Method(s)	Cell Titer Glo, Flow Cytometry
Target Cell Type	MDA-MB-231 adherent and K562 suspension cells
Experiment Plan	Scan plate using Red and Bright field channels
Hypothesis	Determine the counts of PI-positive cells kinetically at 24, 48 and 72 hours

Celigo Setup

Plate Type	Greiner 781091 384-well black wall clear bottom
Scan Channels	Red, Bright field
Resolution	1 μm /pixel
Scan Area	Whole well
Analysis Method	Target 1 + 2
Scan Frequency	Daily, for 3 days
Scan Duration	~15 minutes

Assay Protocol and Plate Setup

Goal: Detect and quantify dead cells using PI stain in adherent MDA-MB-231 and suspension K562 cell lines

Protocol

- Seeded MDA-MB-231 at 2,000 cells/well and allowed to incubate overnight
- Suspension cells were plated the day of experiment with working solution of 2X PI at 3,000 cells/well
- Prepared Benzethonium at 25 μM final concentration and serially diluted by 1.3 dilution factor
- Removed media and added drug, control and PI stain to wells
- Incubated the plate for 24, 48 and 72 hours with drug and dye
- Imaged the plate using the Celigo image cytometer

Plate map for Benzethonium (μM) drug treatment and PI staining

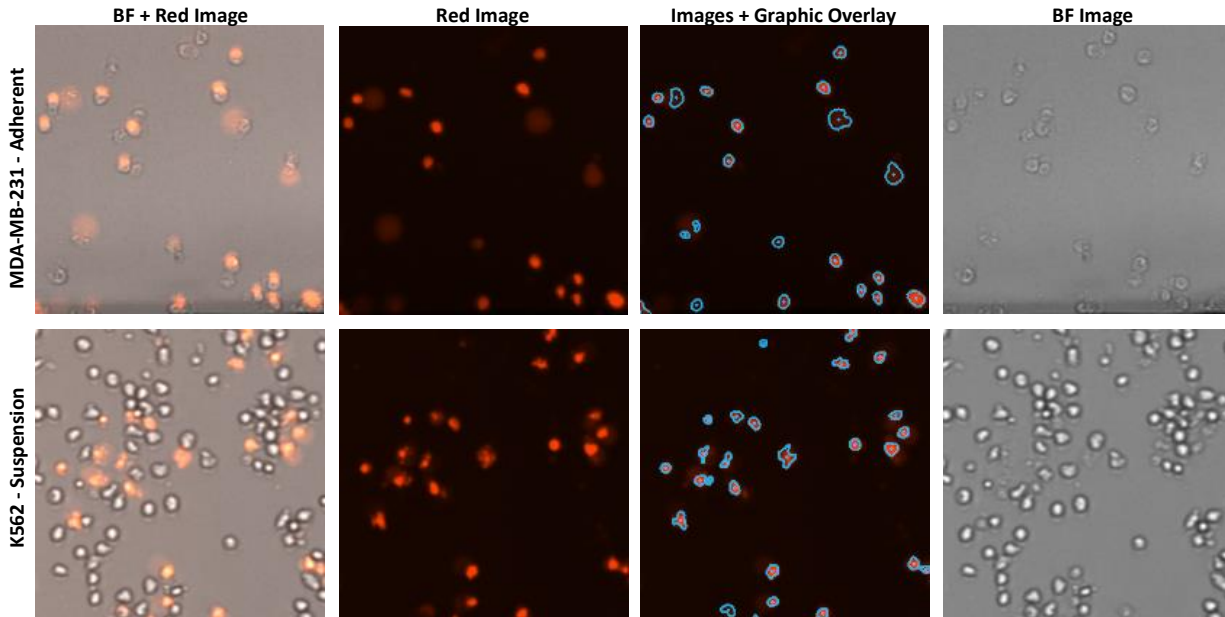
Drug Treatment of Benzethonium (μM)		1	2	3	4	5	6	7	8	9	10	11	12	13
A														
B														
C														
D			25.0	19.2	14.8	11.4	8.8	6.7	5.2	4.0	3.1	2.4	Control	
E														
F														
G														
H			25.0	19.2	14.8	11.4	8.8	6.7	5.2	4.0	3.1	2.4	Control	
I														
J														
K														
L														
M														
N			25.0	19.2	14.8	11.4	8.8	6.7	5.2	4.0	3.1	2.4	Control	
O														
P														

Results

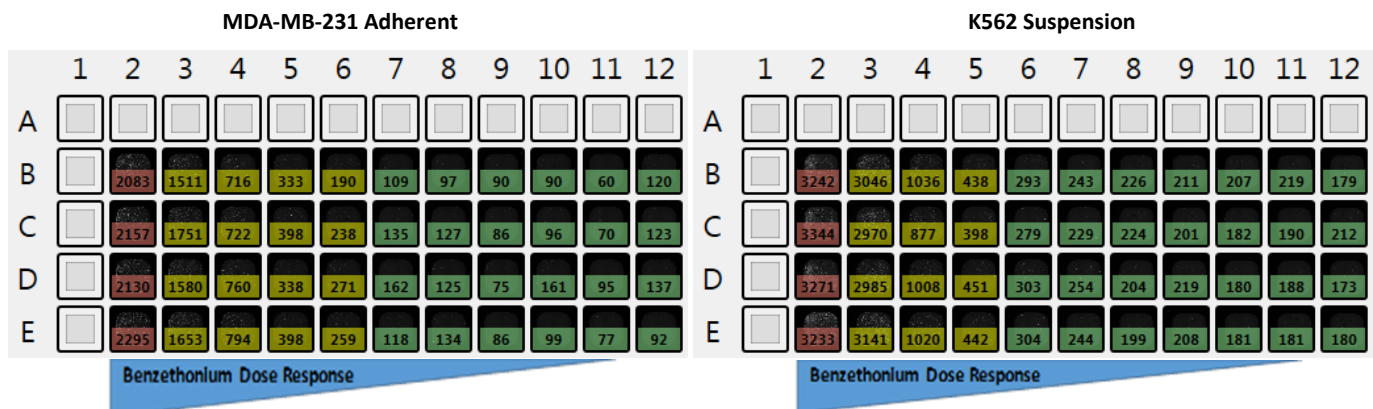
Drug-treated MDA-MB-231 and K562 cells showed an increase in PI positive cells

- PI-positive cells were determined by staining the cells for 24, 48 and 72 hours

Typical images and fluorescent object identification looked as shown below for PI stained cells "Graphic Overlay" segmentation

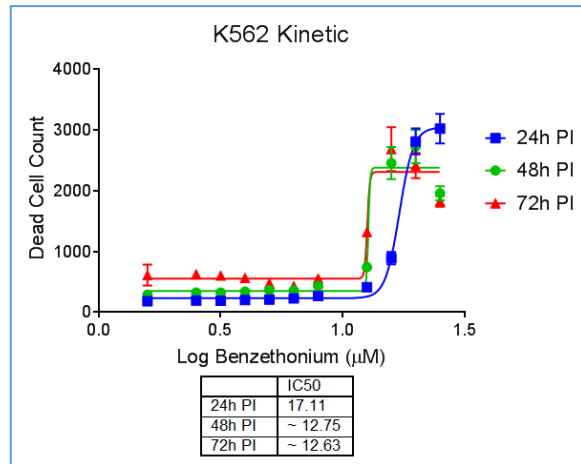
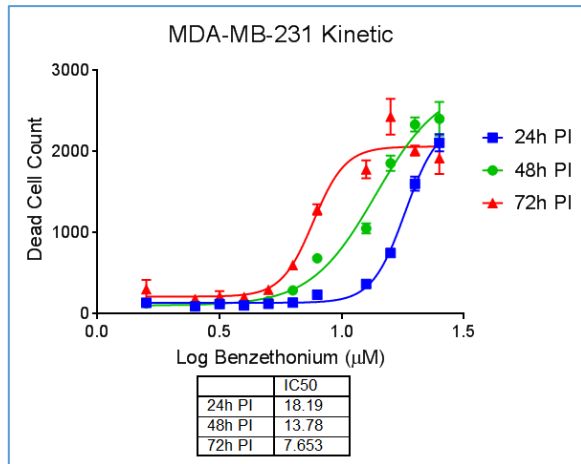


Results for the K562 and MDA-MB-31 counts of dead cells after 24 hours of Benzethonium drug treatment

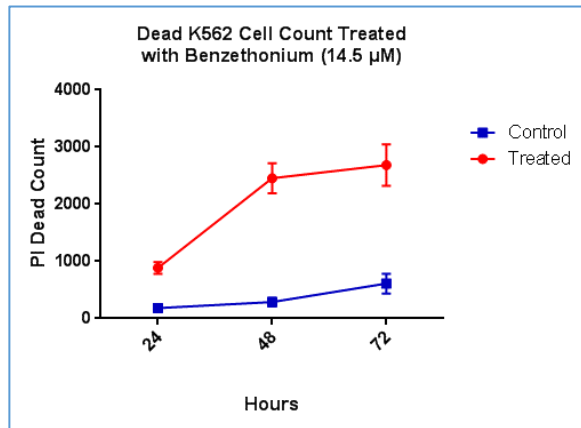
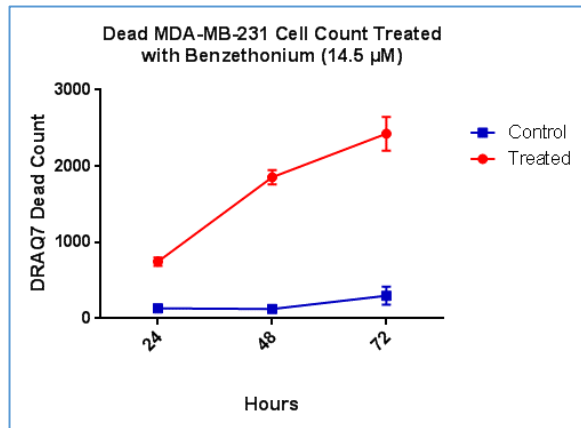


Graphs

- Generated a graph using Microsoft Excel comparing 25 μM Benzethonium to the control after 24, 48 and 72 hours of treatment. In this example, the average of 4 data points were plotted



- IC₅₀ values were calculated with Graph Pad Prism



- Cell death increased over time with Benzethonium (14.5 μM) versus the control

Conclusion

- The Celigo successfully performed PI viability assay using MDA-MB-231 and K562 cell lines drug treated with Benzethonium
- Performed kinetic viability assay using PI allowed for the enumeration of total number of PI-positive cells over a period of 24, 48 and 72 hours