

Assay Name: Kinetic viability using DRAQ7

Assay ID: Celigo_02_0006



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Experiment: Kinetic viability using DRAQ7

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 Far Red and Bright field channels
Hypothesis	Drug treatment will increase the percentage of DRAQ7-positive cells over time

Celigo Setup

Plate Type	Greiner cat# 781091 384-well black wall clear bottom
Scan Channels	Far 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 DRAQ7 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 DRAQ7 at 3,000 cells/well
- Prepared the drug Benzethonium and serially diluted to generate dose response
- Prepared control with water in media
- Removed the media from the wells of adherent cells
- Added drug dose response and control to both adherent and suspension cells
- Added DRAQ7 to adherent cells only
- 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 DRAQ7 time point staining

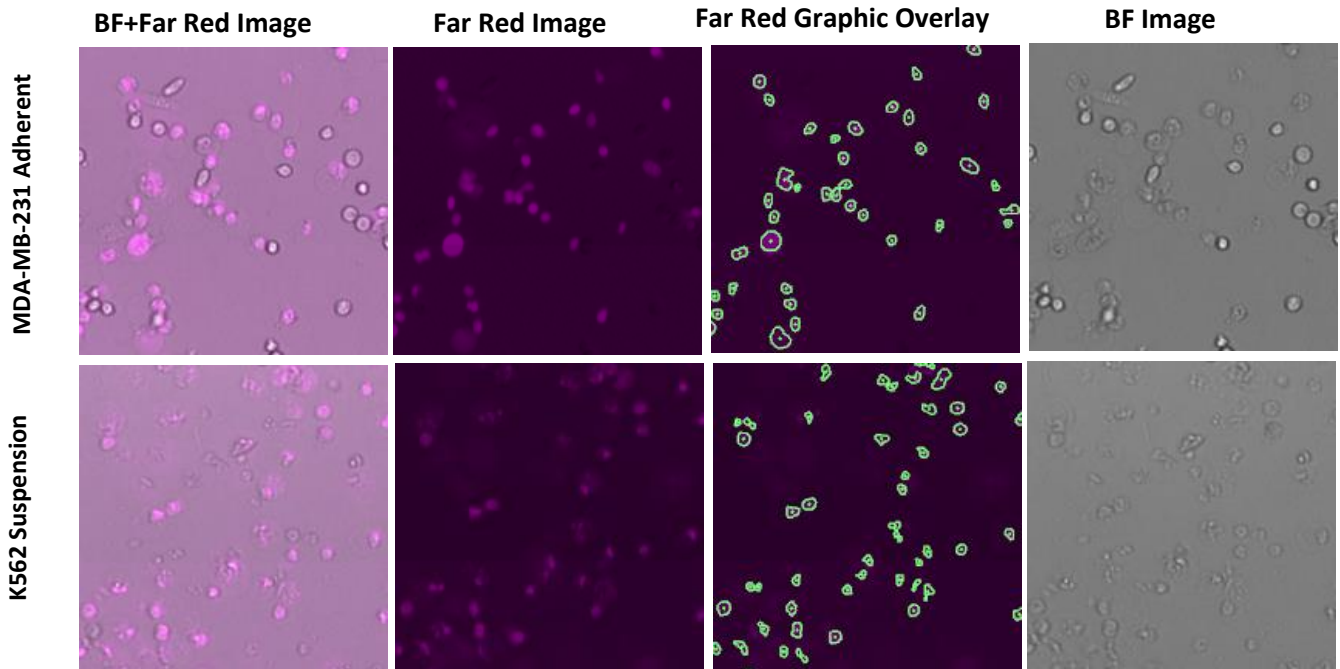
		Drug Treatment of Benzethonium													
		13	14	15	16	17	18	19	20	21	22	23	24		
A															
B															
C															
D		25.0	19.2	14.8	11.4	8.8	6.7	5.2	4.0	3.1	2.4	Control			
E															
F															
G															
H		25.0	19.2	14.8	11.4	8.8	6.7	5.2	4.0	3.1	2.4	Control			
I															
J															
K															
L															
M		25.0	19.2	14.8	11.4	8.8	6.7	5.2	4.0	3.1	2.4	Control			
N															
O															
P															

Results

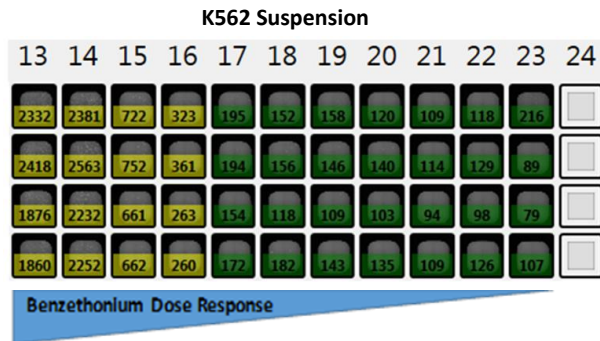
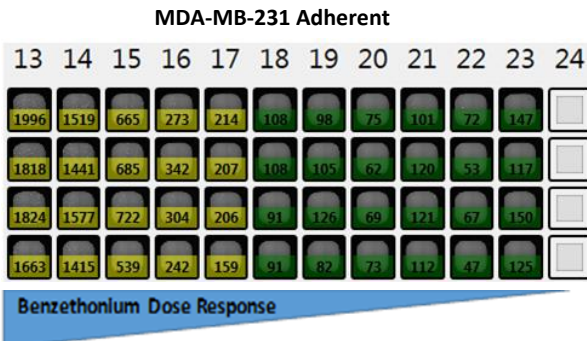
Drug-treated MDA-MB-231 and K562 cells showed an increase in DRAQ7-positive cells

- DRAQ7-positive cells were determined by staining the cells for 24, 48 and 72 hours

Images and fluorescent object identification looked as shown below for DRAQ7-stained cells “Graphic Overlay” segmentation

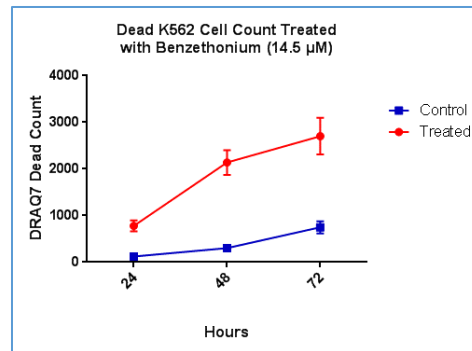
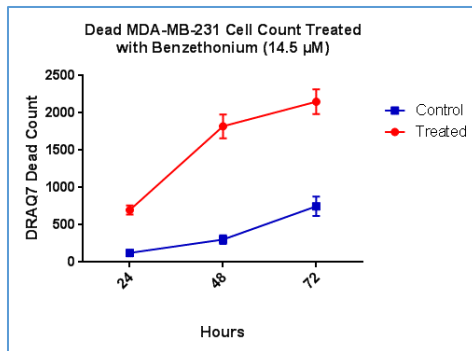
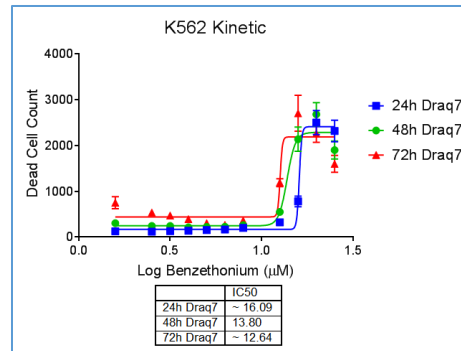
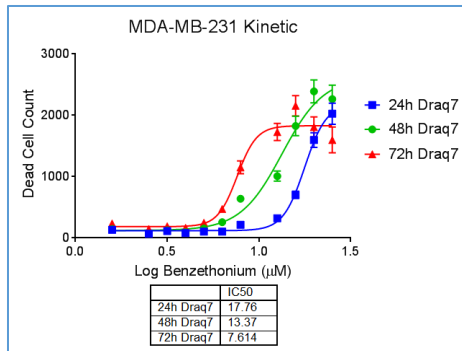


Results for the MDA-MB-31 and K562 counts of dead cells after 24 hours of Benzethonium drug treatment



Graphs

1. Graphs were generated using Graph Pad Prism for the dose response of Benzethonium after 24, 48 and 72 hours treatment. In this experiment, the average of 4 data points were plotted.
2. IC₅₀ values were calculated using Graph Pad Prism
 - Cell death increase over time with Benzethonium (14.5 μM) versus the control



Conclusion

- Drug treated MDA-MB-231 and K562 cell lines were successfully imaged and analyzed on Celigo
- Kinetic viability assay using DRAQ7 allows for the enumeration of total counts of DRAQ7-positive cells over the time
- Acquisition of high resolution DRAQ7 and bright field images of 384-well plate took about 15 minutes