

Assay Name: Endpoint viability using PI and Hoechst

Assay ID: Celigo_02_0010



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Experiment: EndPoint Viability Assay Using PI and Hoechst

Purpose	Perform end point viability assay on MDA-MB-231 and K562 cells treated with Benzethonium for 24, 48 and 72 hours
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, Bright field and Blue channels
Hypothesis	Drug treatment will increase the percentage of PI-positive cells over time

Celigo Setup

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

Assay Protocol and Plate Setup

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

Protocol

- Seeded MDA-MB-231 and allowed to incubate overnight. K562 Suspension cells were plated on the day of the experiment
- Prepared and serially diluted the drug Benzethonium to generate a dose response
- Prepared the control with water in media
- Added drug dose response and control to the wells
- Incubated the plate for 24, 48 and 72 hours
- Prepared a dye mix solution of PI and Hoechst in PBS
- Added dye mix to the drug-treated wells and incubated the plate
- Imaged the plate using Celigo image cytometer

Plate maps for Benzethonium (μM) drug treatment and Hoechst time point staining:

Drug Treatment of Benzethonium (μM)													
	1	2	3	4	5	6	7	8	9	10	11	12	13
A													
B													
C		25.0	19.2	14.8	11.4	8.8	6.7	5.2	4.0	3.1	2.4	Control	
D													
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													

Hoechst Stain Endpoint:													
	1	2	3	4	5	6	7	8	9	10	11	12	13
A													
B													
C		Hoechst added at 24 hr time point											
D		Hoechst added at 24 hr time point											
E		Hoechst added at 24 hr time point											
F		Hoechst added at 24 hr time point											
G		Hoechst added at 48 hr time point											
H		Hoechst added at 48 hr time point											
I		Hoechst added at 48 hr time point											
J		Hoechst added at 48 hr time point											
K		Hoechst added at 48 hr time point											
L		Hoechst added at 72 hr time point											
M		Hoechst added at 72 hr time point											
N		Hoechst added at 72 hr time point											
O		Hoechst added at 72 hr time point											
P		Hoechst added at 72 hr time point											

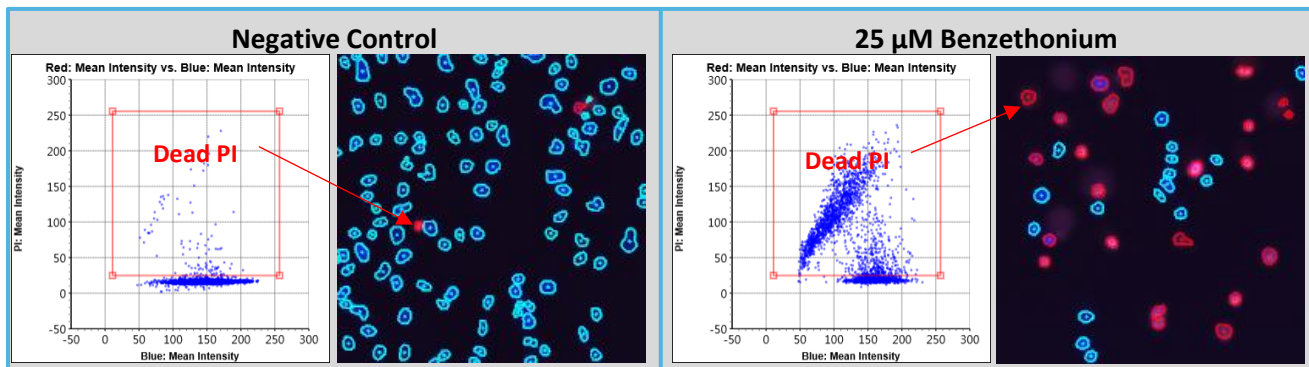
Results

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

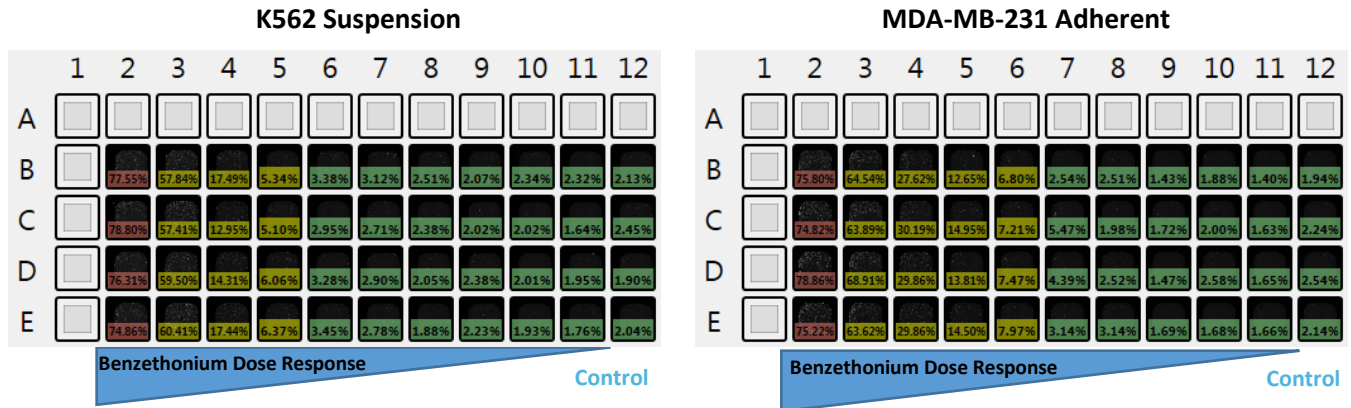
- PI-positive cells were determined by staining the cells at the end point of 24, 48 and 72 hours with PI and Hoechst stains

Gate Plots for PI-Positive Cells:

Example of gating settings for Hoechst + PI stained MDA-MB-231 adherent cell line, with Blue graphic overlay outlining all objects and Red graphic overlay outlining PI-positive cells. Follow a similar setup for work with the suspension cells.

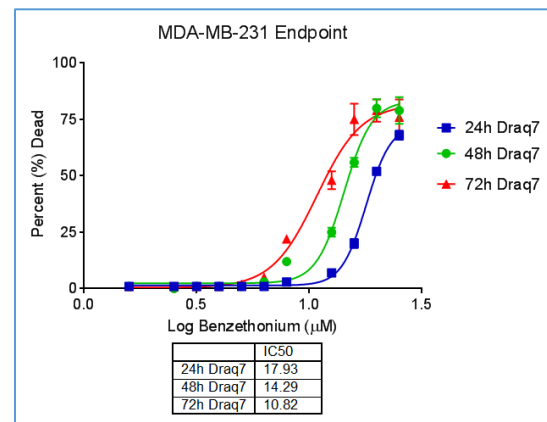
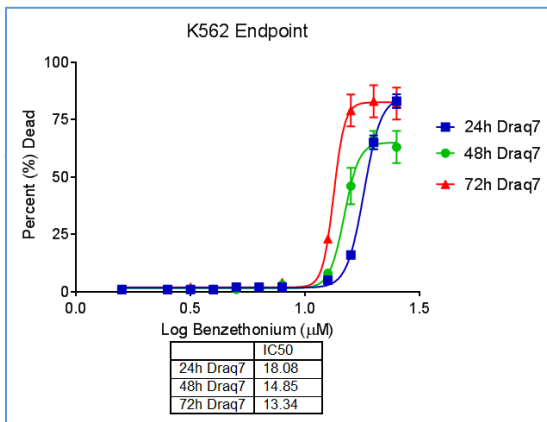


Celigo produced the following results for the MDA-MB-231 and K562 counts of percent dead cells after 24 hours of Benzethonium drug treatment



Graphing

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



- IC₅₀ values were calculated using Graph Pad Prism.

Conclusion

- The Celigo successfully performed PI viability assay using MDA-MB-231 and K562 cell lines
- Performing an endpoint viability assay using PI and Hoechst allowed for the calculation of percentages from the enumeration of PI-positive and total cell counts
- Acquisition of high resolution PI and bright field images of a 384-well plate took ~ 15 minutes