

## Assay Name: Endpoint viability using DRAQ7 and Hoechst

Assay ID: Celigo\_02\_0005



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## Experiment: Endpoint viability using DRAQ7 and Hoechst

Purpose	Perform endpoint 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 Far Red, Bright field and Blue channels
Hypothesis	Drug treatment will increase the percentage of DRAQ7-positive cells

### Celigo Setup

Plate Type	Greiner cat# 781091 384-well black wall clear bottom
Scan Channels	Far Red, Bright field and Blue
Resolution	1 $\mu\text{m}$ /pixel
Scan Area	Whole well
Analysis Method	Target 1 + 2 + Mask
Scan Frequency	Daily, for 3 days
Scan Duration	~15 minutes

### Assay Protocol and Plate Setup

**Goal:** Detect and quantify the dead cells using DRAQ7 and Hoechst total stain in adherent MDA-MB-231 and suspension K562 cell lines

#### Protocol

- Seeded MDA-MB-231 and allowed to incubate overnight. Suspension K562 cells were plated on the first 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 according to the plate map
- Incubated the plate for 24, 48 and 72 hours
- Prepared a dye mix solution of DRAQ7 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 ( $\mu\text{M}$ ) drug treatment and Hoechst time point staining:

		Drug Treatment of Benzethonium												
		13	14	15	16	17	18	19	20	21	22	23	24	
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:											
A													
B		Hoechst added at 24 hr time point											
C													
D													
E													
F													
G		Hoechst added at 48 hr time point											
H													
I													
J													
K													
L		Hoechst added at 72 hr time point											
M													
N													
O													
P													

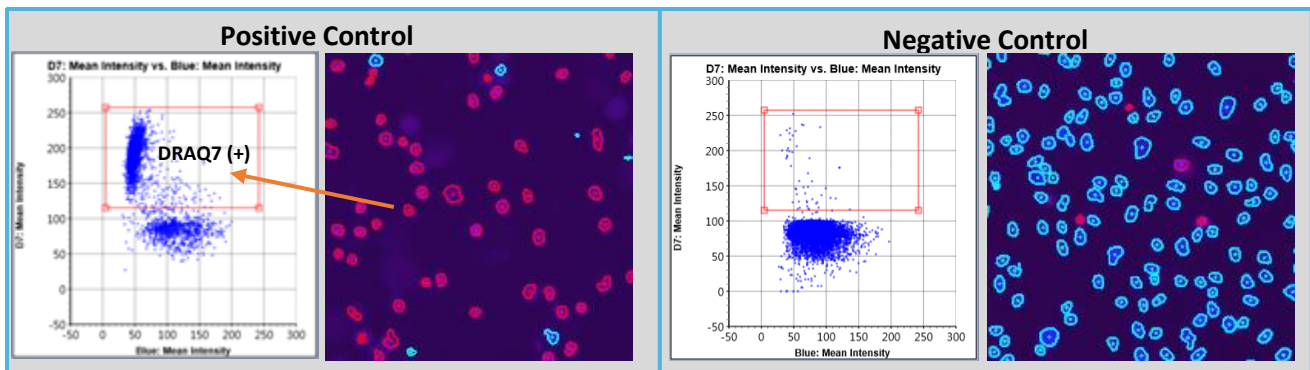
## Results

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

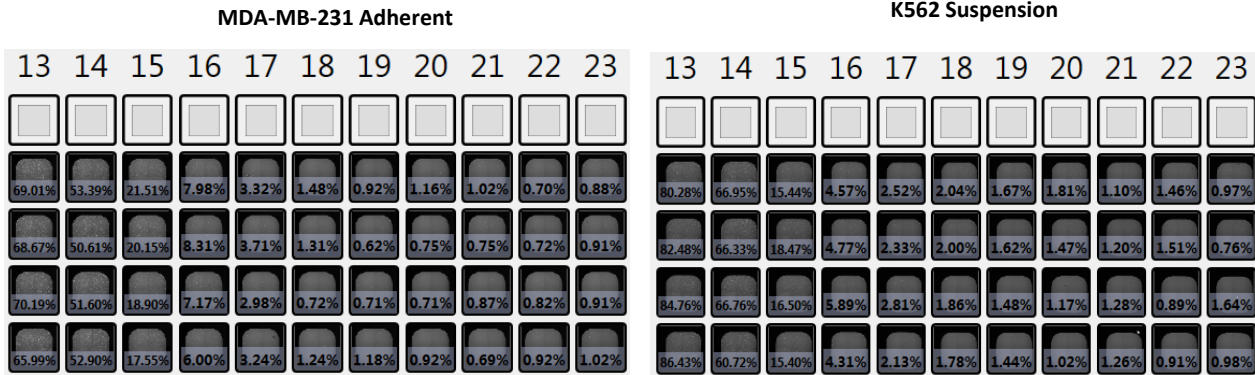
- DRAQ7-positive cells (dead cells) were determined at the endpoint of 24, 48 and 72 hours by DRAQ7 and Hoechst as a total nucleated stain

### Gate Plots for DRAQ7 Positive Cells:

Example of gating settings for Hoechst and DRAQ7-stained MDA-MB-231 adherent cell line, with blue graphic overlay outlining all objects and red graphic overlay outlining DRAQ7 cells. Similar setup was followed to work with suspension cells.

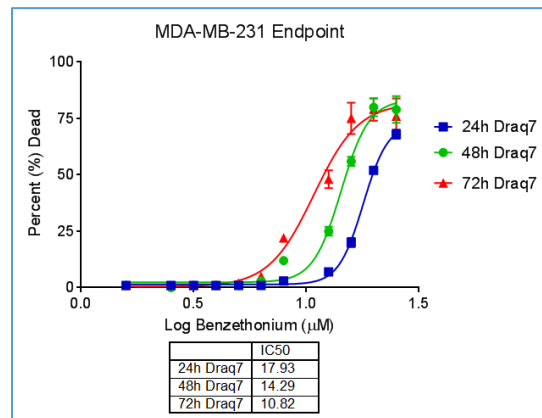
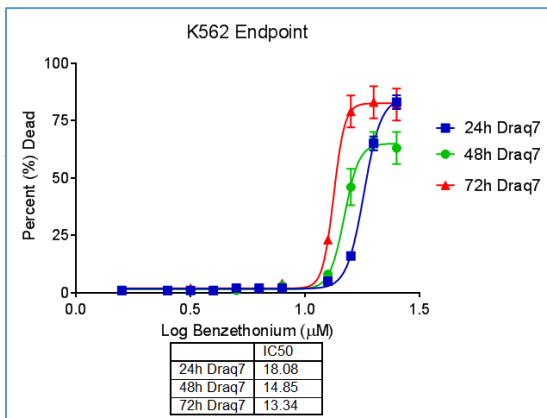


Celigo produced the following results for MDA-MB-231 and K562: percent dead cells after 24 hours of Benzethonium drug treatment



## Graphing

1. Graphs were generated using Graph Pad Prism for the dose response of Benzethonium after 24, 48 and 72 hours of treatment. In this experiment, the average of 4 data points were plotted.
2. The results are shown below:



- IC<sub>50</sub> values were calculated using Graph Pad Prism

## Conclusion

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