Assay Name: Kinetic apoptosis using Caspase 3/7

Assay ID: Celigo_02_0012

Table of Contents

Experiment: Kinetic apoptosis assay using Caspase 3/7

Purpose	Perform apoptosis assay on MDA-MB-231 and Jurkat cells
Current Method(s)	Flow cytometry
Target Cell Type	MDA-MB-231 and Jurkat cells
Experiment Plan	Scan plate using Green and Bright field channels
Hypothesis	By measuring the number of Caspase 3/7 positive cells, we can determine the counts of apoptotic cells in the population

Celigo Setup

Plate Type	96-well Greiner 655090 black wall clear bottom
Scan Channels	Green and Bright field
Resolution	1 µm/pixel
Scan Area	Whole well
Analysis Method	Target 1 + 2
Scan Frequency	0h, 2h, 4h, 6h and 8 hours
Scan Time	~15 minutes

Assay Protocol and Plate Setup

Goal: Detect and quantify apoptotic cells using Caspase 3/7 staining in adherent MDA-MB-231 and suspension Jurkat cell lines

Protocol

- Seeded MDA-MB-231 at 10,000 cells/well and allowed to incubate overnight
- Seeded Jurkat cells at 20,000 cells/well on the day of experiment
- Added Staurosporine at 3 µM final concentration and Caspase 3/7 substrate at 4 µM final concentration per well and allowed to incubate for 8 hours at 37° C
- Imaged the plate every two hours using the Celigo image cytometer for a total of 8 hours

Plate set up

Seeding number of cells/well

		MDA-MB-231						Jurkat				
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		10000	10000	10000	10000			20000	20000	20000	20000	
C		10000	10000	10000	10000			20000	20000	20000	20000	
D		10000	10000	10000	10000			20000	20000	20000	20000	
E		10000	10000	10000	10000			20000	20000	20000	20000	
F		10000	10000	10000	10000			20000	20000	20000	20000	
G		10000	10000	10000	10000			20000	20000	20000	20000	
H												

Drug treatment and control wells

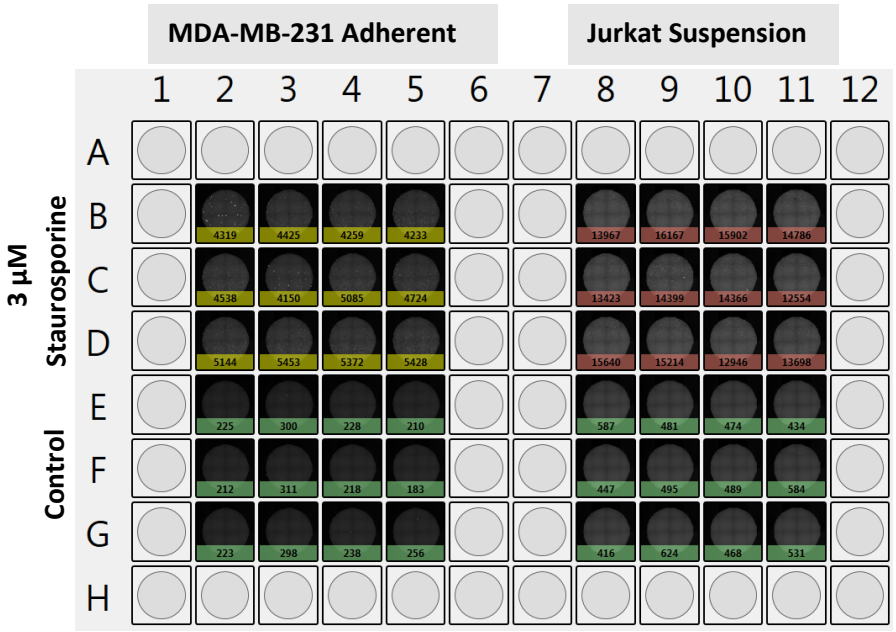
Staurosporine Drug Treatment:												
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		3µM	3µM	3µM	3µM			3µM	3µM	3µM	3µM	
C		3µM	3µM	3µM	3µM			3µM	3µM	3µM	3µM	
D		3µM	3µM	3µM	3µM			3µM	3µM	3µM	3µM	
E		Control	Control	Control	Control			Control	Control	Control	Control	
F		Control	Control	Control	Control			Control	Control	Control	Control	
G		Control	Control	Control	Control			Control	Control	Control	Control	
H												

Results

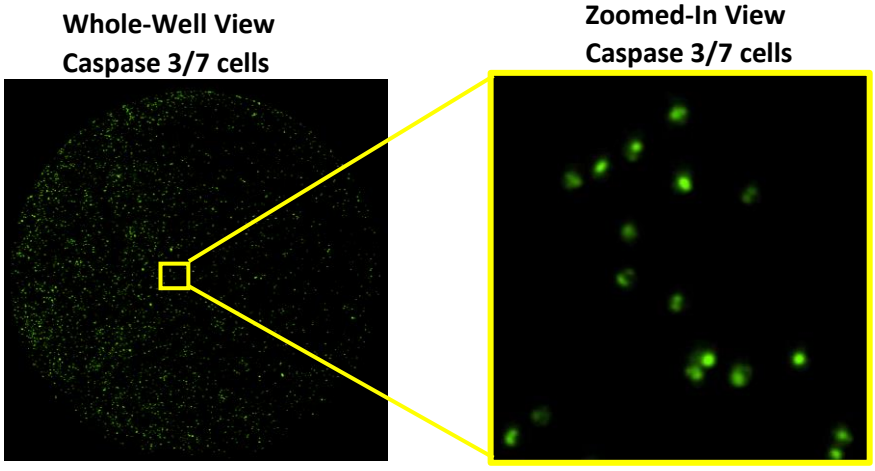
Drug-treated MDA-MB-231 and Jurkat cells showed an increase in Caspase 3/7 positive cells

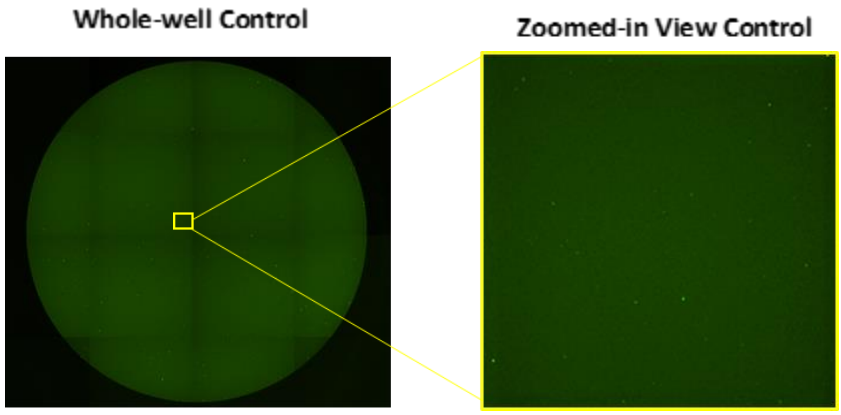
- Bright field images were captured to monitor cell health and morphology
- The total number of apoptotic cells was determined by counting the cells stained with green Caspase 3/7 reagent

Plate-Level View allows for quick observations of the total number of green Caspase 3/7 positive cells. Shown below are typical results of apoptotic (Caspase 3/7 positive) cells after 8 hours of drug treatment.



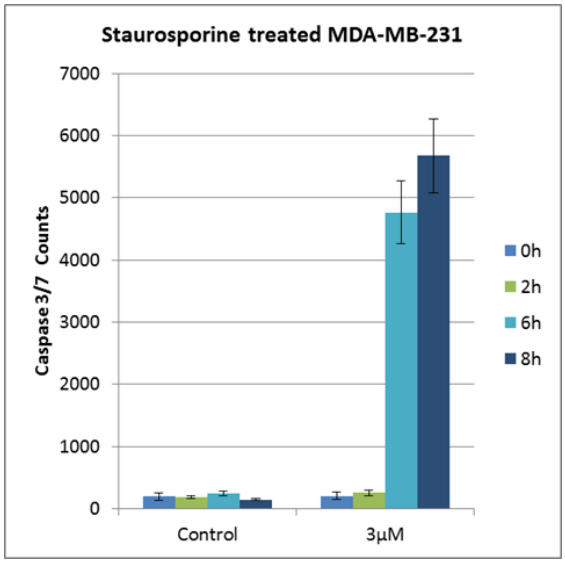
Whole-well view allows for observation of high resolution images .

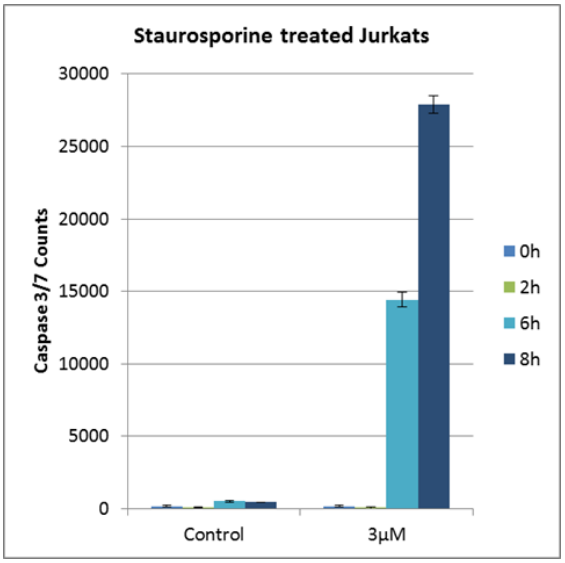




Graphs

1. In Microsoft Excel, create averages and standard deviations of the control and drug-treated wells.
2. Generate a “Bar graph” comparing 3 µM Staurosporine to the control over 8 hour time course. In this example, the average of 12 data points were plotted.





Conclusion

- The Celigo successfully performed Caspase 3/7 apoptosis assay using MDA-MB-231 and Jurkat cell lines
- Acquisition of high resolution bright field and green Caspase 3/7 fluorescent images of an entire 96 well plate took ~ 15 minutes
- Performing kinetic apoptosis assay using Caspase 3/7 allows for the enumeration of Caspase 3/7 positive cells over the time