

Assay Name: Endpoint apoptosis using Caspase 3/7 with Hoechst

Assay ID: Celigo_02_0003

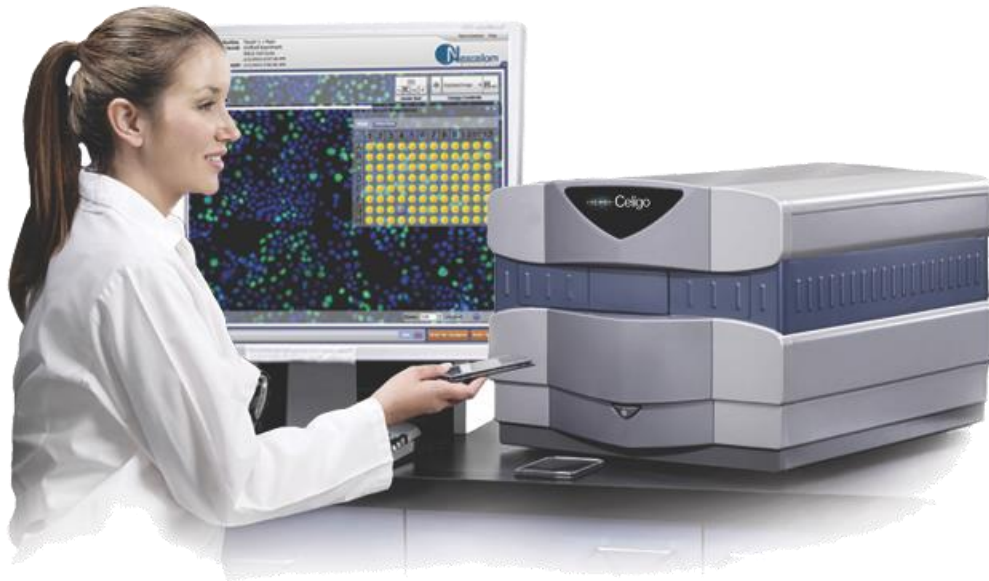


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Experiment: Endpoint apoptosis assay using Caspase 3/7 with Hoechst

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, Bright field and Blue channels
Hypothesis	By measuring the number of Caspase 3/7 positive cells, we can determine the percent apoptotic cells in the population

Celigo Setup

Plate Type	Greiner 655090 96-well black wall clear bottom
Scan Channels	Green, Brightfield and Blue
Resolution	1 μm /pixel
Scan Area	Whole well
Analysis Method	Target 1 + 2 + Mask
Scan Frequency	End Point
Scan Time	~15 minutes

Assay Protocol and Plate Setup

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

Protocol

- Seed MDA-MB-231 at 10,000 cells/well and Jurkat cells at 20,000 cells/well and allow to incubate overnight
- Add Staurosporine at 3 μM final concentration per well and allow to incubate for 4-6 hours
- After incubation is completed, prepare in PBS a 2X concentration of Caspase 3/7 and Hoechst
 - Nexcelom, Cat# CSK-V0003-1
- Remove 100 μL of media from all plate wells.
- Add 100 μL of 2X concentration of Caspase 3/7 and Hoechst and incubate for 30 mins at 37° C
- Image the plate using the Celigo image cytometer

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												

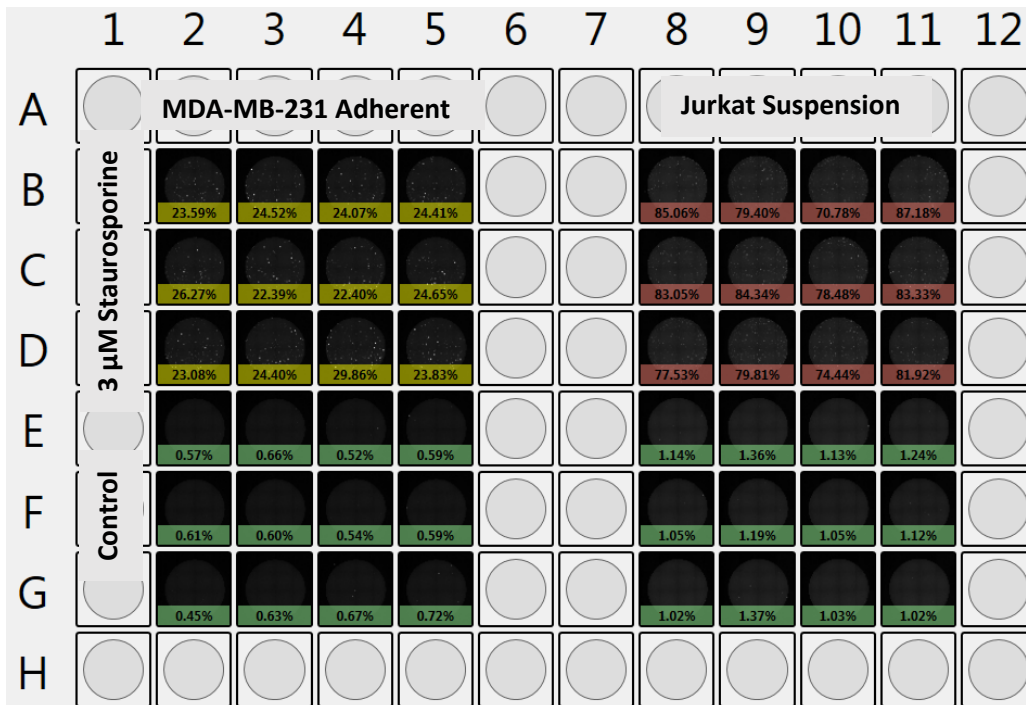
Drug treatment and control wells												
Staurosporin Drug Treatment:												
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	3 μM	3 μM	3 μM	3 μM				3 μM	3 μM	3 μM	3 μM	
C	3 μM	3 μM	3 μM	3 μM				3 μM	3 μM	3 μM	3 μM	
D	3 μM	3 μM	3 μM	3 μM				3 μM	3 μM	3 μM	3 μM	
E	Control	Control	Control	Control	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

Results

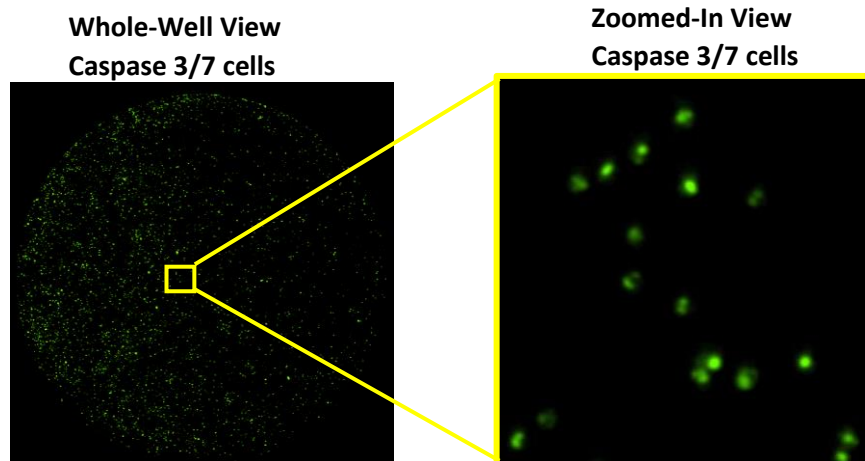
Drug-treated MDA-MB-231 and Jurkat cells showed an increase in Caspase 3/7 positive cells

- Total number of nucleated cells was determined by counterstaining the cells with Hoechst
- Total number of apoptotic cells was determined by counting the nucleated cells stained with green Caspase 3/7 reagent
- Determined the percent of apoptotic-positive cells

Plate-Level Data View allows a quick observation of the total number of cells and green Caspase 3/7 positive cells, as well as percent Caspase 3/7 positive cells. Currently displaying percent Caspase 3/7.



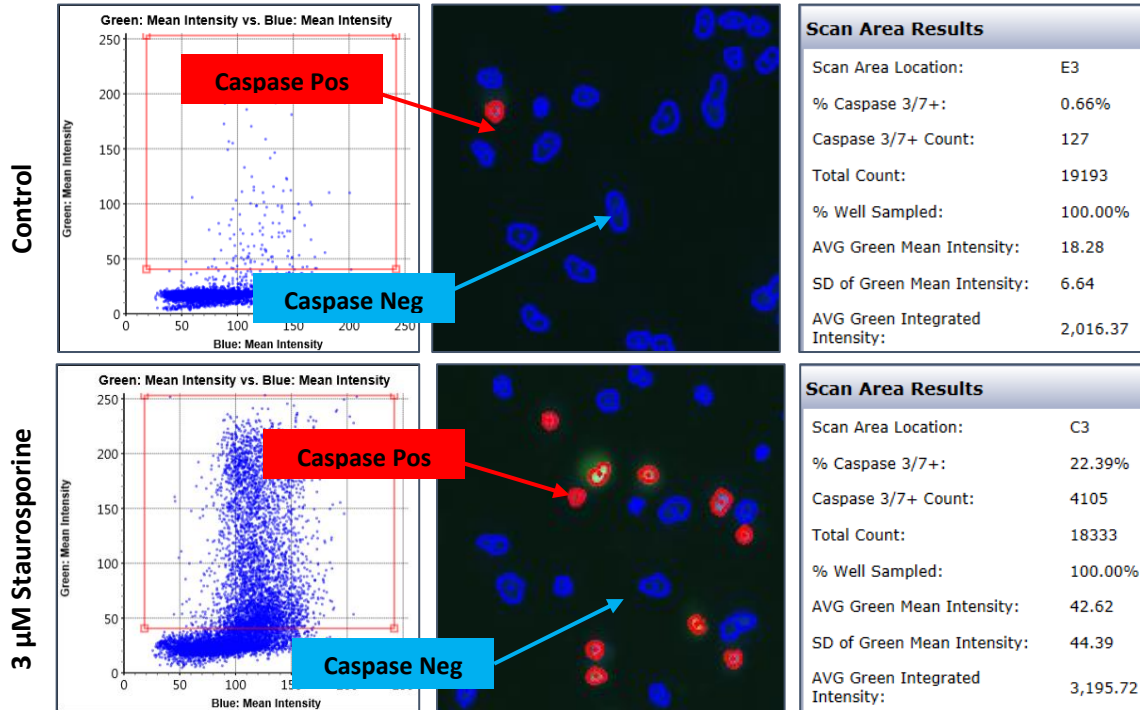
Whole-well view allows high resolution observation of images and at zoomed levels.



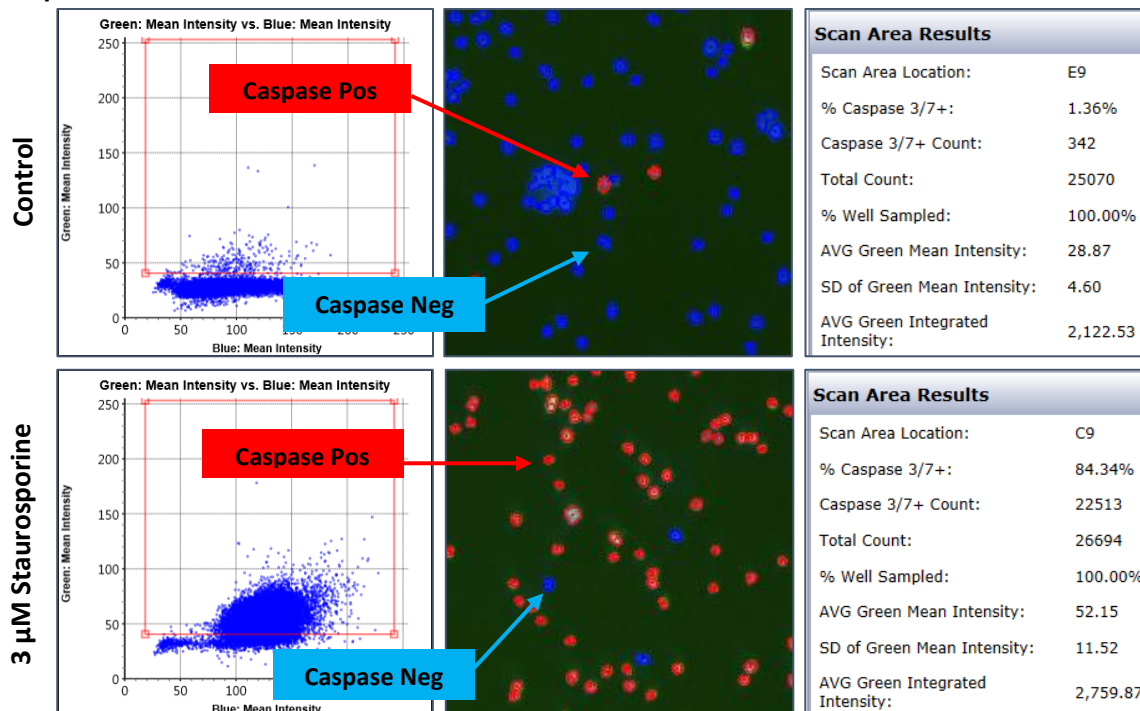
Gate Plots for Caspase 3/7 Positive Cells:

Apoptosis Caspase 3/7 gating analysis of a MDA-MB-231 and Jurkat cells

MDA-MB-231 Adherent:

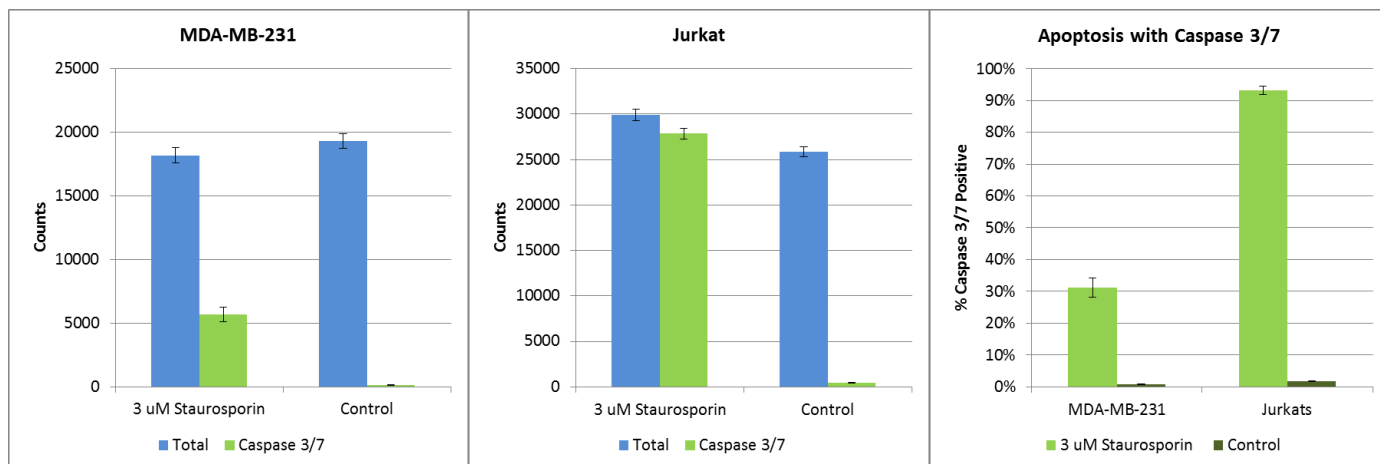


Jurkat Suspension:



Graphs

- Cell counts for adherent, MDA-MBA-231 and suspension, Jurkat cells plotted
- Using Hoechst cell counts as total, percent Caspase 3/7 positive cells are plotted on bar graphs



Conclusion

- The Celigo successfully performed Caspase 3/7 apoptosis assay using MDA-MB-231 and Jurkat cell lines
- Acquisition of high resolution bright field, Caspase 3/7, and Hoechst fluorescent images of an entire 96 well plate took ~ 15 minutes
- Performing an endpoint apoptosis assay using Caspase 3/7 with Hoechst allows for the enumeration of the total number of nucleated cells and total number of Caspase 3/7 positive cells, as well as to determine the percent of apoptosis.