Assay Name: Kinetic apoptosis using Caspase 3/7

Assay ID: Celigo_02_0012

Description: Kinetic detection of apoptosis in adherent and suspension cells using Nexcelom ViaStain™ Live Caspase 3/7 and bright field imaging.

Stains: Nexcelom ViaStain™ Live Caspase 3/7 detection for 2D/3D Culture

Imaging channels: Green and Bright field

Image analysis algorithm: Celigo software Expression Analysis: Target 1 + 2

Methods:

Adherent cells (MDA-MB-231)

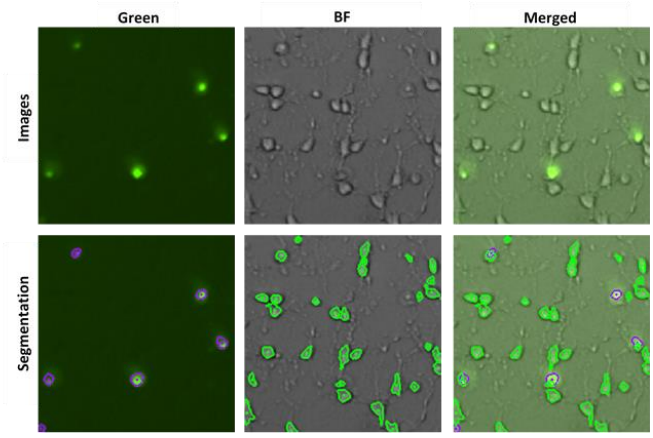
1. Seed 10,000 cells/well in 96-well plate and incubate cells overnight
2. Remove media and add Caspase 3/7 as well as Staurosporine or vehicle control
3. Image at time point 0 and then incubate
4. Image at 2h, 6h and 8h on the Celigo image cytometer

Suspension cells (Jurkat)

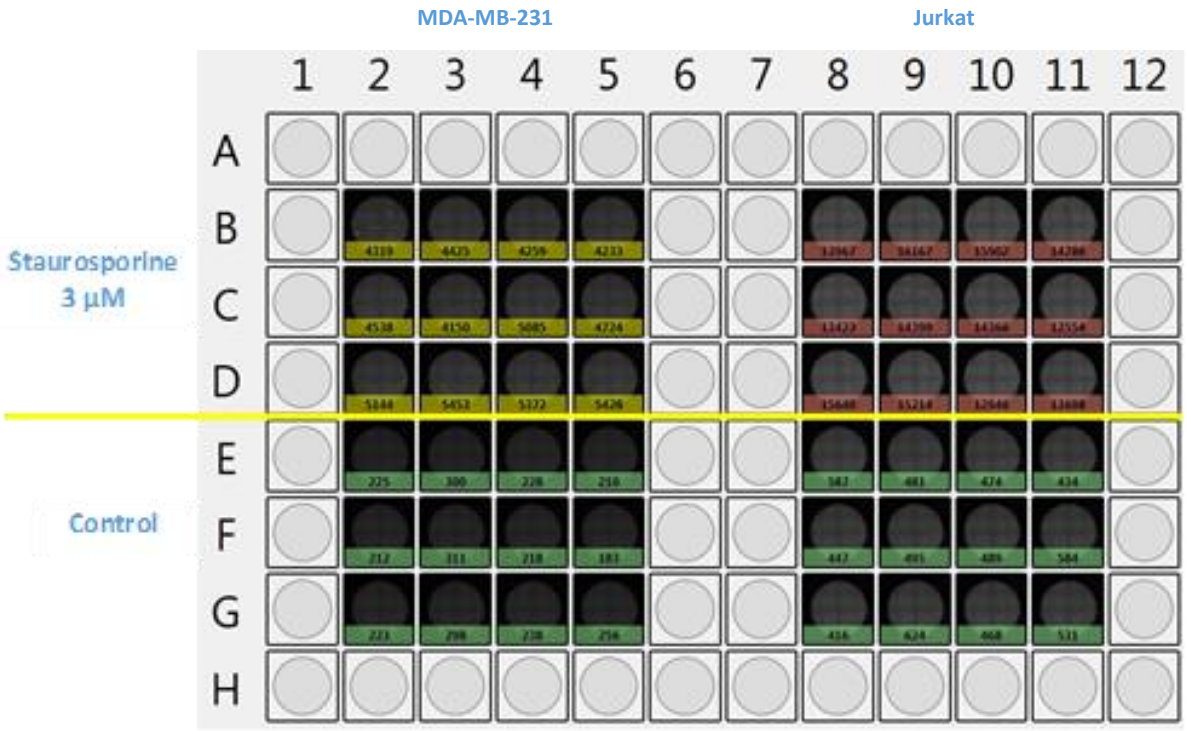
1. Prepare cell suspension solution of 200 cells/µL in media and add Caspase 3/7
2. Seed 20,000 cells/well in 96-well plate
3. Add 2x Staurosporine (apoptotic inducing drug) or the vehicle control
4. Image at time point 0 and then incubate
5. Image at 2h, 6h and 8h on the Celigo image cytometer

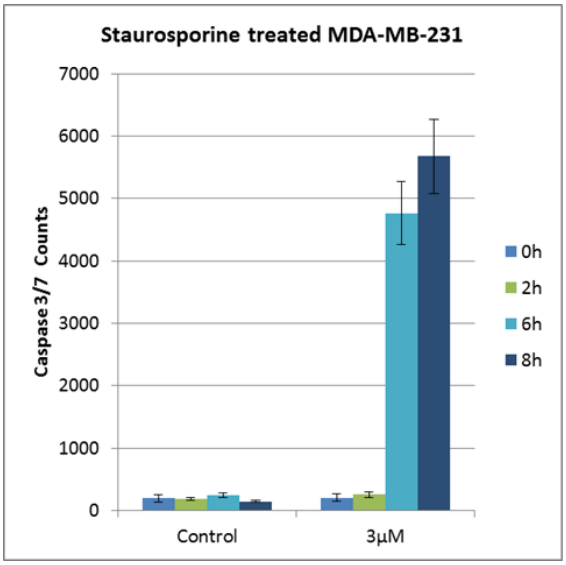
Results:

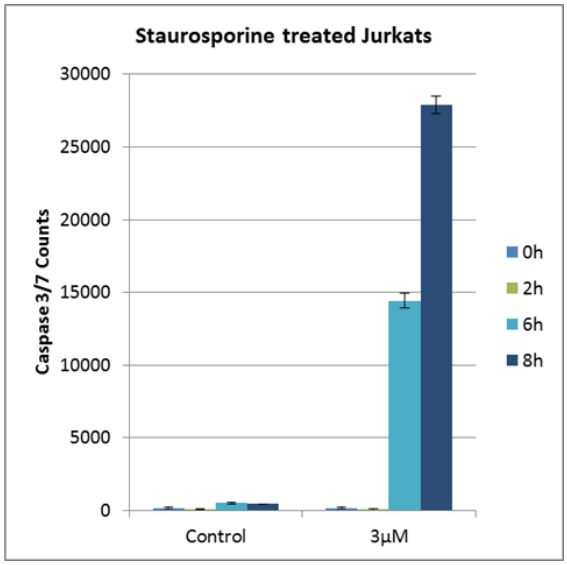
Detection of apoptosis in MDA-MB-231 and Jurkat cell using Caspase 3/7



- Typical images of Caspase 3/7 labelled apoptotic MDA-MB-231 cells treated with Staurosporine, with or without segmentation, to allow cell identification.







- Whole-plate readout with the heat map applied. Green for wells containing a low number of apoptotic cells, yellow for wells with an intermediate level of apoptotic cells and red for a well containing a high number of apoptotic cells
- The Nexcelom ViaStain™ Live Caspase 3/7 detection for 2D/3D Culture can kinetically track the induction of apoptosis by Staurosporine in both MDA- MB-231, an adherent cell line, and Jurkat, a suspension cell line.