Assay Name: Cell cycle analysis using DAPI and BrdU

Assay ID: Celigo_02_0001

Description: Analysis of cell cycle using DAPI and BrdU incorporation on A549 cells

Stains: Alexa Fluor® 488 goat anti-mouse antibody and DAPI

Imaging channels: Green and Blue

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

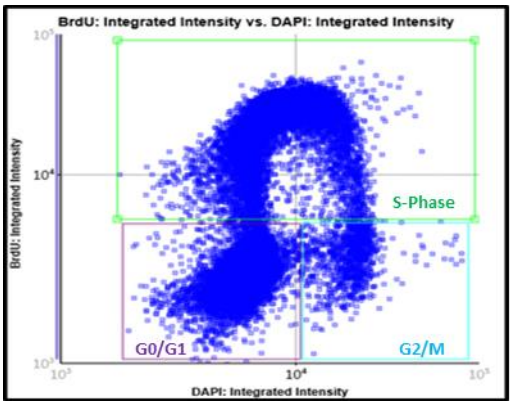
Methods:

1. Seed cells in 96-well plate and incubate cells overnight
2. Add 100 µl of compound at 2x desired final concentration (e.g. Aphidicolin, an inhibitor of DNA synthesis)
3. BrdU incorporation step, 100 µM BrdU and incubate for 2 hours
4. Fixation and DNA denaturation step, 200 µl/well of Ethanol/Glycine solution for 30 minutes
5. Antibody labeling steps:
    a. 50 µl Anti-BrdU mouse monoclonal antibody and incubate for 2 hr
    b. 50 µl Anti-BrdU Alexa Fluor® 488 goat anti-mouse antibody and incubate for 1 hr and wash
6. Add 100 µl DAPI mix to each well and incubate for 30 min
7. Image and gate on the Celigo

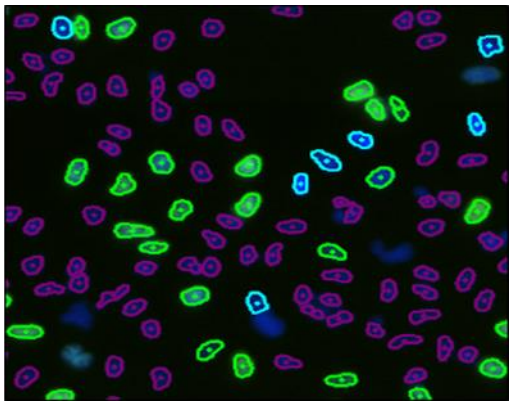
Results:

Gating interface displaying the horseshoe plot and cells are color-coded dependent on the phase of the cell cycle they are in.

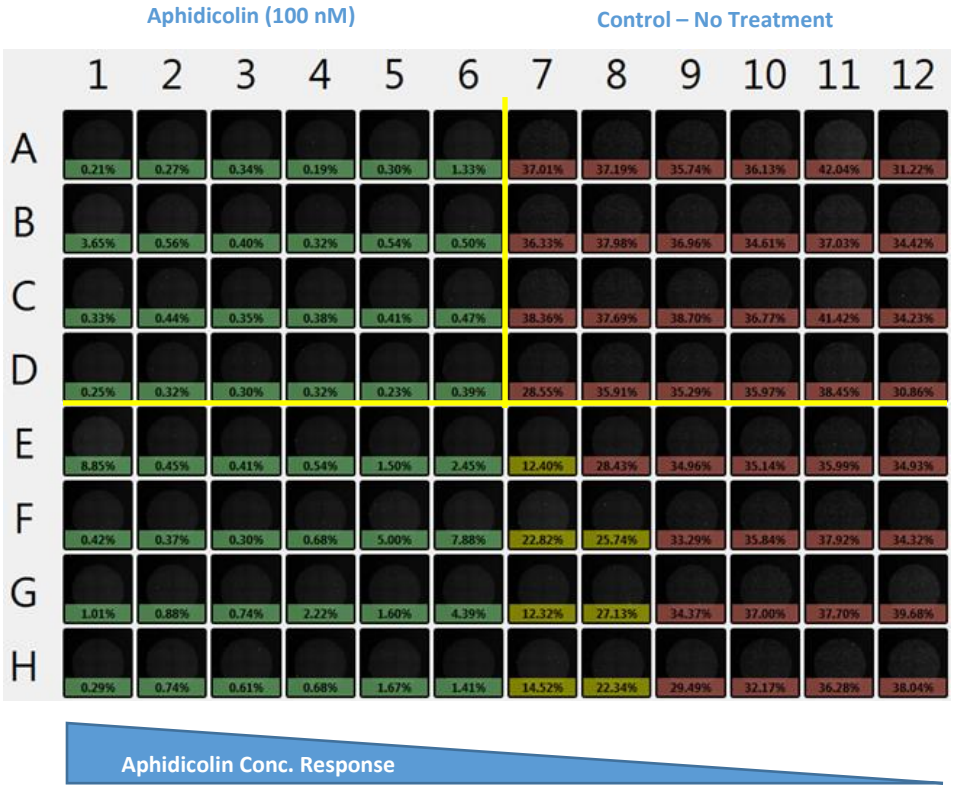
Gating Interface Plot



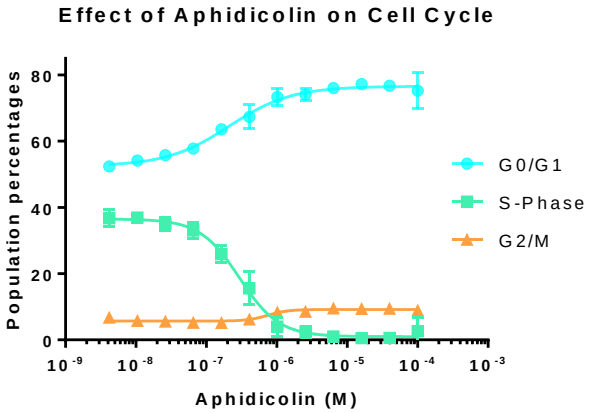
Cell Segmentation



- BrdU integrated intensities vs DAPI integrated intensities are plotted to create the horseshoe shape in the above left image.
- S-phase cells are identified in green, G0/G1 cells in purple and G2/M in blue in a control well in the above right image.



- 96 well plate readout displaying % cells in S-phase and a heat map display.
- The top half of the plate contains 100nM Aphidicolin and controls; the bottom is a serial dilution of Aphidicolin across the plate.
- In the heat map, green represents low % cells in S-Phase, yellow and intermediate % cells in S-phase and red is a high % of cells in S-phase.



	G0/G1	S-Phase	G2/M
IC50	2.11e-007	2.952e-007	7.449e-007