APPLICATION NOTE

The CellSimple Cell Analyzer:

A fast simple dye-based assay for measuring cell health.

Introduction to the CellSimple Cell Analyzer

Complex cell and bead-based assays are made simple and fast with the CellSimple™ Cell Analyzer. This instrument combines a 488 nm laser, dual photomultiplier tubes (PMT), Coulter Principle-based cell measurements and on-board software to provide easy-to-run applications and data analysis. Moreover, the instrument relies on disposable cassettes for sample handling, which alleviates the need for flow cell cleaning and fluidics maintenance. And, the instrument is small enough to be portable between the lab bench and the hood.

When paired with highly validated kits and reagents from Cell Signaling Technology (CST), the CellSimple Cell Analyzer enables powerful plug-and-play assays, such as measuring the health of a cell culture sample.

Advantages of the CellSimple Cell Analyzer	
Fast	*Accurate results in less than a minute right at your bench!*
Powerful	*Bead and cell-based experiments at the push of a button.*
Simple	*No fluidics or flow cell to maintain. No extensive training to operate.*
Portable	*Small size enables movement between the lab bench and the hood.*
Affordable	*Priced to make complex cellular analysis routine.*

Background and Experimental Design:

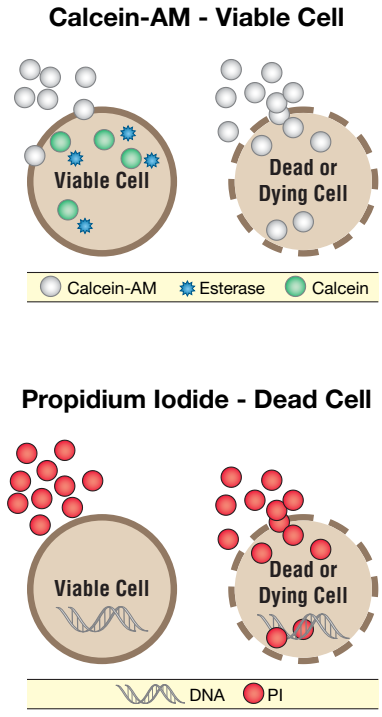
Measuring the viability of cells in culture is critical when performing cell-based assays as the health of the culture can have a dramatic impact on the overall success of the experiment. There are several ways to measure cell viability, but one common method relies on dyes that specifically label either living or dead cells. Calcein-AM and Propidium Iodide (PI) are two dyes that are routinely used in these types of assays.

Calcein-AM is a membrane permeant molecule that provides a good estimation of living cells. Once inside the cell it is converted into a green-fluorescent calcein dye after removal of the AM (acetoxymethyl ester) by esterase; an enzyme only active in living cells.

Conversely, PI is a bright red, fluorescent dye that binds to DNA, but is unable to permeate the intact membrane of living cells. This feature makes it useful for measuring cytolysis or cellular membrane leakage, a hallmark of dead and dying cells.

Here we use Calcein-AM, PI and the Cell Health Application on the CellSimple Cell Analyzer to determine the viability of a cell culture sample in a single assay in response to the cytotoxic agent staurosporine.

Figure 1: Experimental Design



The CellSimple Cell Analyzer

Methods:

Culture Conditions:
Jurkat cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum at 37°C in 5% CO_2.

Treatments:
Cells were either left untreated or treated with staurosporine (10 µM, 18 hr).

Sample Preparation and Analysis:
Unfixed cells were stained with PI (0.5 µM) and Calcein-AM (0.05 µM) in a single reaction tube. Mean fluorescent intensity (MFI), cell number, and percent of total population were analyzed using the Cell Health Application setting of the CellSimple™ Cell Analyzer as described in the Users Manual.

Results:

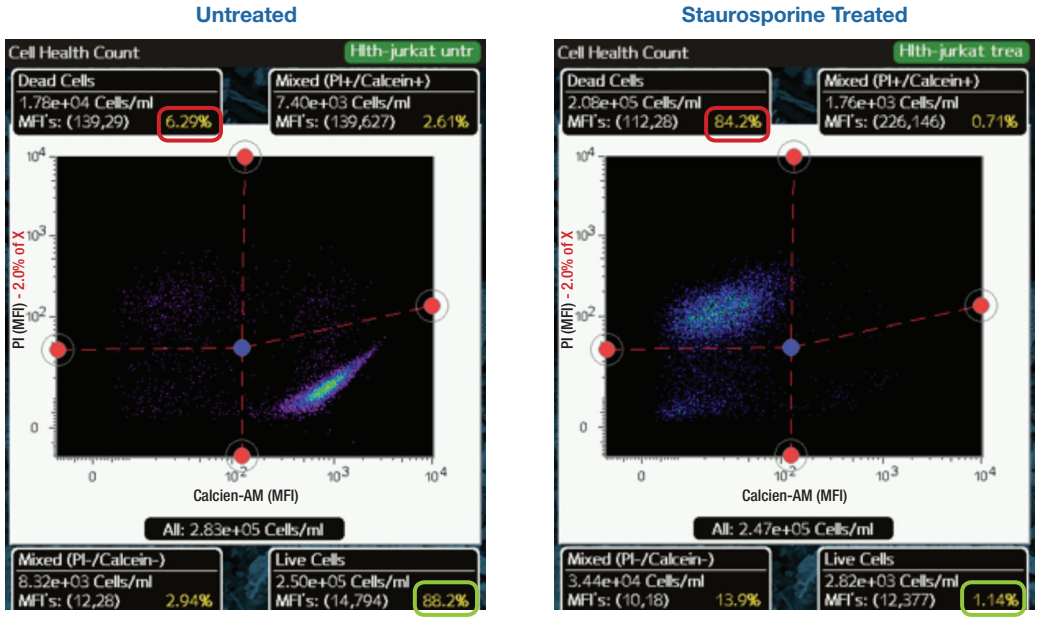


Figure 2:
Jurkat cells were either untreated (left panel) or treated with staurosporine (right panel). The cell count, MFI and and percent of total population for each panel can be visualized at the corner of each gate.

Treatment with staurosporine induces cell death, shifting the cellular population from the lower right hand quadrant (live cells) to the upper left hand quadrant (dead or dying cells) of the plot **(Figure 2)**. The Cell Health Application on the CellSimple Cell Analyzer automatically runs data analysis to quantify the shift, revealing a drop in living cells from 88.2% in the untreated culture **(left, green circle)** to 1.14% in the staurosporine-treated culture **(right, green circle)** and an increase in dead cells from 6.29% in the untreated culture **(left, red circle)** to 84.2% in the staurosporine-treated culture **(right, red circle)**.

Conclusion:

These data demonstrate that the Cell Health application on the CellSimple Cell Analyzer used in conjunction with Calcein-AM and PI in one assay allows a researcher to measure the health of a cell sample quickly and accurately.

Importantly, the CellSimple Cell Analyzer can be paired with other cellular dyes, allowing investigators to measure parameters such as apoptosis, cell viability, mitochondrial potential, and cell proliferation, among others. This provides investigators with the flexibility to develop assays to assess the overall health of their cell cultures. Not only are these assays powerful, but with the CellSimple Cell Analyzer they are also rapid, accurate, affordable, and performed right at your lab bench.

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Cell Signaling TECHNOLOGY®