APPLICATION NOTE

The CellSimple Cell Analyzer:

A simple, rapid method for simultaneously measuring the activity of two pathways.

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 independent changes in pathway activation.

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:

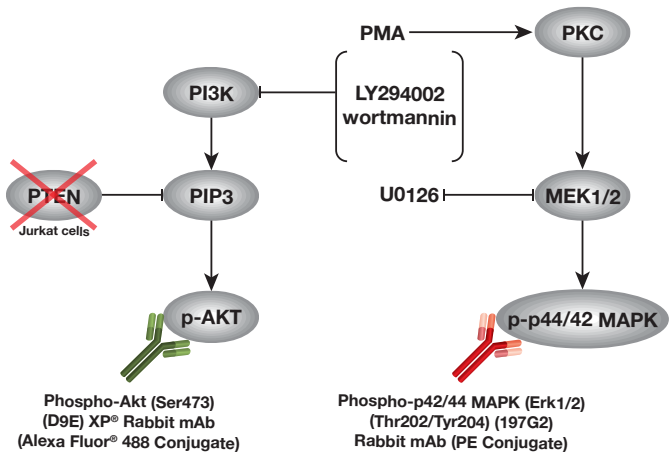
The PI3 Kinase (PI3K)/Akt and MAP kinase (MAPK) pathways are critically important signaling pathways that act to translate information from the extracellular environment into cellular behavior, such as cell proliferation, survival, differentiation and motility.

Akt and p42/44 MAPK (ERK) serve as integration hubs for their respective pathways and their activation (via phosphorylation) can be used as a reliable proxy of pathway activity.

The PI3K/Akt pathway is constitutively active in Jurkat cells due, at least in part, to the loss of the negative pathway regulator PTEN (Shan, et al., 2000). Pathway activity can be attenuated, however, with inhibitors to PI3 Kinase (LY294002 and wortmannin). In contrast, the basal activity of MAPK pathway is low in Jurkat cells, but can be stimulated by treatment with phorbol-12-myristate-13-acetate (PMA, a.k.a. TPA), which activates PKC and initiates a cascade resulting in p44/42 MAPK (ERK) phosphorylation. PMA-induced activation of the MAPK pathway can be blocked by pretreating the cells with U0126, which targets MEK1 and MEK2 and prevents PKC-induced ERK phosphorylation **(Figure 1)**.

Here we show that the CellSimple Cell Analyzer can be paired with phospho-specific antibodies from CST to simultaneously measure the activity of the PI3K/Akt and MAPK pathways, in response to chemical modulation, in a single cell.

Figure 1: Experimental Design



The CellSimple Cell Analyzer

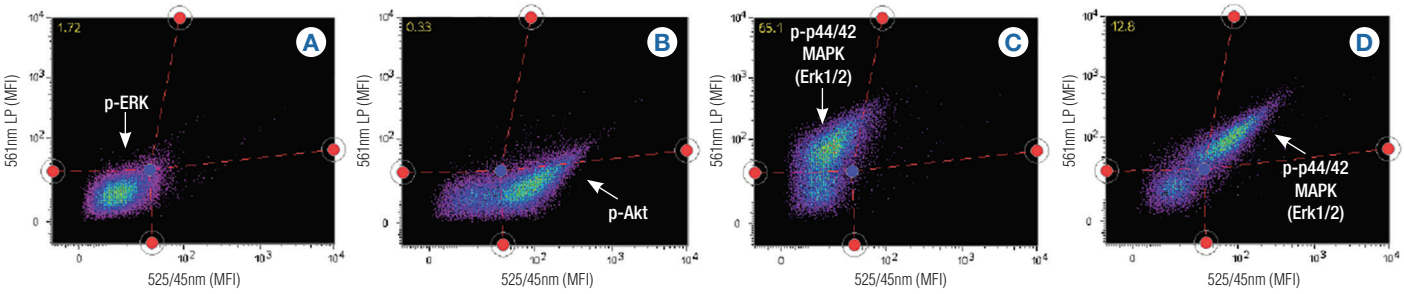
Methods:

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

Treatments:
LY294002 was used at a concentration of 10 µM for 2.5 hr. Wortmannin was used at a concentration of 1 µM for 2.5 hours. U0126 was used at a concentration of 2 µM for 2.5 hr. PMA was used at a concentration of 1 µM for 15 min. PMA was added at the end of the LY294002/wortmannin incubation period, when used in combination with other chemical modulators.

Sample Preparation and Analysis:
Formaldehyde fixation and methanol permeabilization were performed according to the standard CST protocol for intracellular flow-cytometry. The cells were then double-stained with Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (197G2) Rabbit mAb (PE Conjugate) #14095, and Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #4071, using standard CST protocols for immunofluorescent analysis. The samples were analyzed using the Open Flow Application of the CellSimple™ Cell Analyzer as described in the CellSimple Users Manual. Fluorescence emission from phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (197G2) Rabbit mAb (PE Conjugate) was measured using PMT1 (561 nm/LP) and Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) was measured using PMT2 (525/45 nm). Please note standard CST protocols can be found at www.cellsignal.com.

Results:



Panel	Treatment	Pathway Status	Result
A	LY294002, wortmannin, U0126	PI-3K/Akt = Off MAPK = Off	Neither Akt nor p44/42 MAPK (Erk1/2) is phosphorylated, so the fluorescent signal in both channels is low.
B	U0126	PI-3K/Akt = On MAPK = Off	Akt is phosphorylated, so the fluorescent signal in PMT2 (x-axis) is high. p44/42 MAPK (Erk1/2) is unphosphorylated, so the fluorescent signal in PMT1 (y-axis) is low.
C	(LY294002, wortmannin) > PMA	PI-3K/Akt = Off MAPK = On	Akt is unphosphorylated, so the fluorescent signal in PMT2 (x-axis) is low. At the same time, p44/42 MAPK (Erk1/2) is phosphorylated, so the fluorescent signal in PMT1 (y-axis) is high.
D	PMA	PI-3K/Akt = On MAPK = On	Both Akt and p44/42 MAPK (Erk1/2) are phosphorylated, so the fluorescent signal in both channels is high.

Conclusion:

These data indicate that the CellSimple Cell Analyzer can be paired with antibodies against phosphorylated proteins to measure independent activity changes in multiple signaling pathways at the cellular level.

More broadly, the CellSimple Cell Analyzer can be paired with any number of fluorescently labeled antibodies, allowing investigators to measure the expression of individual proteins at the cellular level. This provides investigators with the flexibility to develop powerful assays for unraveling the interplay between components of a single signaling cascade or between the components of interconnected pathways. 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.

References:

Shan, et al. *Mol Cell Biol.* 2000 Sep; 20(18): 6945–6957.

www.cellsignal.com/cellsimple

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