Rapid, cell-based *in situ* cell health assessments

Introduction

Assays of cell health are the most common assays performed in cell biology. Cell health assays are fundamental to early drug safety evaluation in drug discovery as well as in general maintenance of cells in culture and basic research. Cell health assays fall generally into the categories of cell proliferation, cell stress, apoptosis, and toxicity. Most of these types of assays are usually performed using plate readers without access to cell-level information. By contrast, many high content analysis systems include cell health assays which do provide cell-level data using high resolution analysis, but are time consuming and require significant training to execute. The Celigo cytometer is an easy alternative platform for a wide variety of cell health assays providing cell-level information while maintaining the simplicity of conventional plate reader assays.

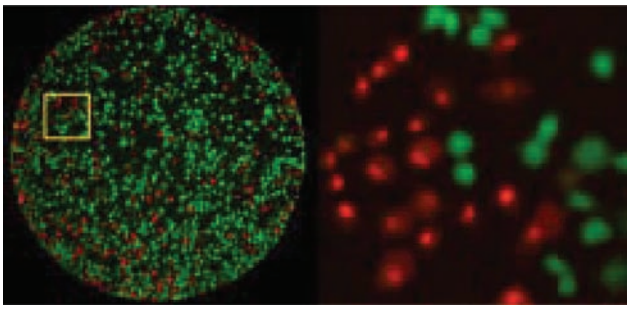

Fig. 1 Image of entire well of a 384-well plate. Celigo's unique optics image entire wells of microplates of various sizes (6-well to 384-well plate). The left panel shows an overlay image of a 384-well where cells stained with calcein (green) and propidium iodide (red). The square yellow insert is magnified in the right image, highlighting the ability to discriminate the size and morphology of live green cells compared to the red nuclear stain of dead cells.

The Celigo™ Cytometer is a microplate-based brightfield and fluorescent imaging system designed to rapidly visualize, identify, and quantify cell-based assays at the single cell level. Celigo enables fast imaging of entire wells (Fig. 1) of microplates (6-well to 384-well plates) while maintaining consistent illumination and contrast out to the well edge for accurate identification of all cells within the well. This application note demonstrates use of several fluorescent cell health assays on Celigo.

Approach and Results

Cell Viability (Toxicity)

The cell viability assay is a method for studying cytotoxicity, cell health, and cell death. The fluorescent viability assay is based on the simultaneous detection of live and/or dead cells with probes that reflect cellular activities and plasma membrane integrity. Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, which can be determined by the enzymatic conversion of the virtually non-fluorescent cell-permeable calcein AM to the polyanionic dye calcein that is well retained within live cells and produces an intense cellular fluorescence. Late-apoptotic, necrotic, and dead cells are characterized by having a compromised plasma membrane. The altered plasma membrane is rendered permeable to many dyes, such as propidium iodide, which can be used to selectively stain dying cells.

Briefly, cells are simultaneously stained with a mixture of calcein AM, propidium iodide, and Hoechst 33342 for respectively staining of live, dead and all cells. Images are acquired and analyzed using the Celigo software. Markers are identified in each fluorescent channel and for each well of a microtiter plate, live and dead cell counts as well as the percentage of live and dead cells are automatically reported.

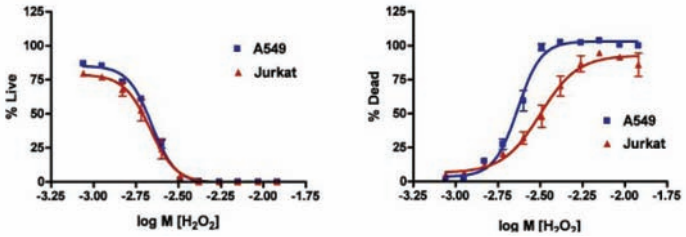

Fig. 2 Cell viability measurement with Celigo. A549 and Jurkat cells were treated with H2O2 in a concentration range from 0.1 to 12 µM for 4 hr and assessed for viability using the Celigo Cell Viability application.

Fig. 2 shows representative data from a typical cell viability experiment on Celigo using adherent A549 and non-adherent Jurkat cells. Percentages of live and dead cells were automatically calculated and adjusted for total cell counts. As the concentration of hydrogen peroxide increases, we observed a decrease in live cell percentage (left) and an increase in dead cell percentage (right) for both cell types. A549 cells exhibited calculated IC50 and EC50 values of 2.2 mM for both live and dead percentages whereas Jurkat cells exhibited calculated values of 2.2 mM and 3.2 mM for percentage live cells and percentage dead cells, respectively.

PS Externalization (Apoptosis)

The Annexin V-based apoptosis detection assay is a method for studying apoptosis that detects changes in the position of phosphatidylserine (PS) in the cell membrane. In non-apoptotic cells, most PS molecules are localized at the inner layer of the plasma membrane, but soon after inducing apoptosis, PS redistributes to the outer layer of the membrane and becomes exposed to the extracellular environment. PS translocation precedes other apoptotic events, thus allowing early detection of apoptosis. Exposed PS can be easily detected with annexin V, a 35.8-kDa protein that has a strong affinity for PS.

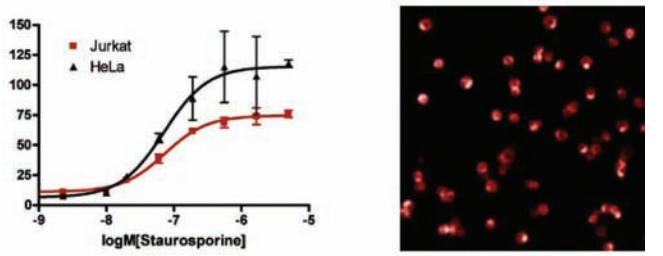

Fig. 3 PS Externalization (Apoptosis) measurement with Celigo. HeLa and Jurkat cells were treated with staurosporine in a concentration range from 0.5 to 5000 nM for 18 hr and assessed for osphatidylserine (PS) externalization using the Celigo PS Externalization application.

Apoptotic cells were stained with fluorescently-conjugated annexin V, a "PS Externalized" cell stain, and "total" cell stain (i.e., Hoechst 33342). Cells were imaged in the corresponding fluorescent channels on Celigo and analyzed to determine the number of apoptotic ("PS Externalized") cells. To validate the PS Externalization Application, adherent HeLa and non-adherent Jurkat cells were treated with staurosporine to induce apoptosis. The Celigo cytometer was used to determine the percentage of cells that positively stained for annexin V or "PS Externalization" positive. PS externalization positive cells were automatically calculated and adjusted for total cell counts. As expected, the levels of PS externalization positive cells increased in a concentration-dependent fashion for both cell lines tested. Calculated EC50 values were 73 nM and 72 nM for HeLa and Jurkat cell lines, respectively.

DNA Synthesis (Cell Proliferation)

Cell proliferation and the characterization of agents that either promote or inhibit cell proliferation are particularly critical areas of cell biology and drug-discovery research. 5-bromo-2´-deoxyuridine (BrdU), a thymidine analog, can be used to detect DNA replication in actively proliferating cells. Cells which have incorporated BrdU into DNA can be quickly detected by using monoclonal antibodies directed against BrdU and fluorescently-conjugated secondary antibody. BrdU staining facilitates the identification of cells that have progressed into or through the S-phase of the cell cycle during the BrdU-labeling period.

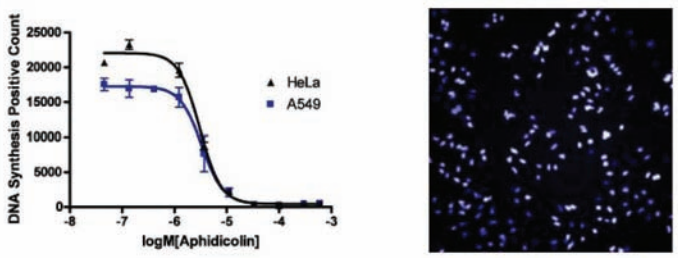

Fig. 4 DNA Synthesis measurement with Celigo. HeLa and A549 cells were treated with aphidicolin in a concentration range from 0.05 to 600 µM for 18 hr and assessed for cell proliferation (DNA Synthesis) using the Celigo DNA Synthesis application.

The Celigo DNA Synthesis application was used for rapid full well imaging and accurate identification and quantifcation of cells undergoing DNA synthesis. Briefly, live cells were pulse labeled with BrdU, cells were fixed, and BrdU was detected by immunofluorescence. The resulting stained cell classes were BrdU positive cells or "DNA Synthesis Positive", and a "total" cells (Hoechst 33342). Fig 4 shows representative data from a cell proliferation assay on Celigo using adherent A549 and HeLa cells. The number of DNA Synthesis positive cells was automatically calculated. Calculated IC50 values were 3.1 and 3.3 µM for A549 and HeLa cells, respectively.

Conclusion

These data demonstrate the ability of the Celigo cytometer to accurately determine experimental effects on cells by measuring proliferation, viability, and apoptosis. The ability to carry out these varied applications for cell-based research as well as to provide a platform for optimizing and maintaining cell cultures streamlines workflow and increases efficiency for any laboratory performing cell based research. Celigo provides a simple, rapid, low-cost alternative to current methodologies for measuring cell health.

References

1. Sasaki et al. Flow cytometric estimation of cell cycle parameters using a monoclonal antibody to bromodeoxyuridine. Cytometry. 1986; 7(4):391-5.

Nexcelom Bioscience
360 Merrimack Street
Building 9
Lawrence, MA 01843, USA
T: +1.978.327.5340
F: 978.327.5341
www.nexcelom.com