Multiplexing Cell-Based Assays using the SpectraMax® i3 Multi-Mode Microplate Reader with MiniMax™ Imaging Cytometer

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Abstract

Complex cell-based assays are developed to provide more biologically relevant and predictive experiments. The SpectraMax® i3 Multi-Mode Microplate Platform with MiniMax™ Imaging Cytometer enables multiplexed analysis of important biological outputs from cell-based assays. The spectrometers are designed for environments from basic research to screening, and the intuitive user interface allows non-imaging specialists to get up and running quickly. We demonstrate how this platform allows researchers to obtain more biologically relevant data by combining imaging with plate reader assays. Quality control assessment of the cells in a microplate can be performed before running an in vitro assay on the same platform. Cardiomyocyte beating can be measured and compared with cell viability data, and cell signaling can be examined through a combination of readouts to improve assay development. Plate reader assays using luminescence, HTRF®, or other detection modes can easily be combined with imaging cytometry to obtain more information on the mechanisms of cell toxicity.

Using cellular imaging to normalize assay data

Here a luminescence apoptosis assay was performed in combination with cellular imaging to assess viability and determine degree of confluence in each well prior to assay. HeLa cells were plated at 2500, 5000, and 10,000 cells per well in a 96-well plate, representing differences in cell density that can result from mis-pipetting or variability in cell growth from well to well. Cells were treated with DMSO (control) or with 37 μM staurosporine for 4 hours to induce apoptosis. They were then stained fluorescently with calcine AM to determine the cell viability, and the percent area covered by cells was determined using the MiniMax Imaging Cytometer. Afterwards, the very same cells were assayed for caspase activity using the luminescent Caspase-Glo 3/7 Assay (Promega) and were detected using the luminescence detection mode of the SpectraMax i3 Platform. The top panel shows caspase assay data (RUU) normalized to the cell covered area imaging data for all three cell densities. For each cell density, the normalized numbers are similar, indicating a similar caspase response regardless of cell density within the range tested. By normalizing the luminescence data to the imaging data, we demonstrate how we are able to correct for differences in cell density that could result from plating error or variability in cell growth across the microplate. The bottom panel shows an additional quality control measure: bright-field images of control and staurosporine-treated cells using the transmitted light (TL) detection mode of the MiniMax Imaging Cytometer.

Combining fluorescent and luminescent readouts for apoptosis and cell viability

Here cells were treated to induce apoptosis and assayed for caspase activity and cell viability. HeLa cells were plated at 5000 cells per well in 384-well black-well, clear-bottom microplates and allowed to grow overnight. The following day, a serial dilution of anisomycin was added to the cells. After 20 hours, apoptosis and cell viability were measured.

Apoptosis was monitored using the CellEvent Caspase 3/7 Green Detection Reagent (Life Technologies), which produces a green fluorescent signal in apoptotic cells where caspase-3 or caspase-7 has been activated. Apoptotic cells were quantified using the SpectraMax® i3 MiniMax Imaging Cytometer with Cell Count analysis (top images). Bright-field images show rounding of the anisomycin-treated cells, consistent with apoptosis (bottom images). Cell viability was quantitated using the CellTiter-Glo Luminescent Cell Viability assay (Promega) and detected with the SpectraMax i3 Platform. Normalized results for both assays are shown in the graph above. All data were collected and analyzed using SoftMax® Pro Software.

Cardiotoxicity assessment, continued

A combination of cellular imaging and HTRF®(Cubis) was used here to assess cell signaling in Beta-2 adrenergic receptor-transfected cells. Different transfection conditions were evaluated. The TransFec® Assay ( Molecular Devices) was used to image cell signaling. With this technology, arrestin-GFP is dispersed throughout the cytosol in unstimulated cells. Upon ligand binding with the GPβ3, the arrestin-GFP translocates from the cytosol to the cell membrane and then into clathrin-coated pits at the cell membrane and then into clathrin-coated pits at the cell membrane. The receptor then internalizes into endocytic vesicles prior to reprocessing and returns to the cell membrane. Vesicles in beta-2 adrenergic receptor-transfected, isoproterenol-treated cells were imaged and quantified using the MiniMax Imaging Cytometer (top panels). The HTRF®-cAMP dynamic 2 assay was used as an additional means to monitor cell signaling and confirm the best transfection condition. The relative levels of cAMP were shown to correlate closely with vesicle count for the different transfection conditions tested.

Conclusions

• The SpectraMax® i3 Multi-Mode Microplate Platform with MiniMax Imaging Cytometer option allows users to couple measurements of cell viability with images to reader and imaging cytometry modes, providing you with more biologically relevant data.

• The ability to combine a general cell health assay with a measurement of a specific effect increases confidence in the quality of your toxicity results.