Application Note

Cell Health and Viability

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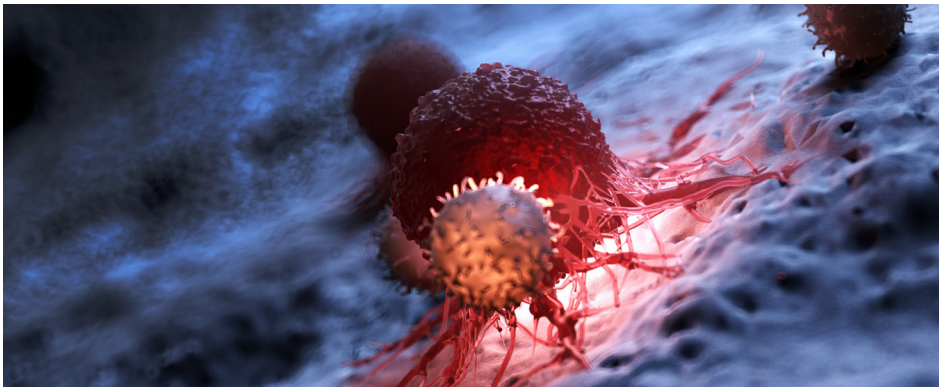
Automated Cell Viability Assay

Automation of liquid and microplate handling and microplate reading



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Abstract

Automated systems capable of performing liquid and microplate handling and microplate reading have revolutionized the field of cell-based assays by streamlining processes, improving accuracy, and increasing throughput. This study highlights the automation of a homogeneous cell-based assay that is commonly used for assessing cell viability and cytotoxicity via a luminescent readout.

Introduction

Automated high-throughput screening (HTS) cell proliferation and cytotoxicity assays are designed for use in multiwell microplate formats. A homogeneous, single-reagent luminescent assay is a widely used method for quantifying the number of viable cells in these assays based on their metabolic activity. This assay relies on the measurement of adenosine triphosphate (ATP), an indicator of metabolically active cells. As viable cells produce ATP, its measurement provides valuable insight into cell health and response to various treatments. Automating the assay offers several advantages: 1) automated systems ensure consistent reagent delivery and mixing, reducing human error and therefore increasing precision and accuracy; 2) multiple samples can be processed simultaneously, significantly increasing assay throughput; 3) automation reduces hands-on time, allowing researchers to focus on data analysis and interpretation; and 4) smaller volumes of reagents are required, making it cost-effective and reducing waste.

Automation of a luminescent assay requires a liquid handler to aspirate media and dispense reagents consistently and with a high degree of precision. Minimizing the amount of reagent required can be accomplished by aspirating a portion of the media, leaving a known residual volume. Reagent is then added at a ratio of 1:1. A microplate reader is used to measure luminescence, which correlates with ATP levels in the samples, and a microplate handler is used to transfer microplates between the liquid handler and microplate reader. Automated cell seeding, addition of test compounds, and other treatment into microplates can also be accomplished based on throughput needs. Following treatment, the cells are incubated as per the experimental protocol. The reagent is automatically dispensed into each well and the plate is typically shaken to allow for cell lysis to occur and the luminescence reaction to begin. The plate is then transferred to the microplate reader and read. Available reagents with extended half life allow the flexibility for batch-mode processing of a large number of microplates.

The microplate reader quantifies the luminescent signal and software is used to analyze the data. Cell viability is determined based on the luminescence values obtained and correlates with the number of viable cells: higher luminescence indicates more viable cells, while lower luminescence indicates fewer viable cells. Researchers can use this data to assess the effects of treatments, screen for compounds, or evaluate cytotoxicity.

Experimental

Instrumentation

Agilent BioTek 406 FX washer dispenser

The Agilent BioTek 406 FX washer dispenser builds on the capability of its predecessor, the Agilent BioTek EL406 washer dispenser. The 406 FX is an automated microplate processor that can perform microplate washing steps in 96-, 384-, and 1536-well microplates. In addition to standard wash routines, the 406 FX has built-in cell-washing capabilities. An internal buffer switching valve allows for the selection of up to four different wash buffers without changing bottles. A built-in sonicator provides the capability for automated cleaning maintenance of the dispense manifold. The device has up to six different reagent dispensers. Two separate peristaltic pumps capable of dispensing from 1 to 3,000 µL. Different cassettes (1-, 5-, and 10 µL) provide accuracy at different dispense volumes while minimizing priming volume. The 406 FX can also be configured with up to two additional syringe modules, each with two independent syringe-pump dispensers capable of dispensing from 5 to 3,000 µL. The 406 FX is capable of plate shaking at three different speeds and is robotic compatible.

Figure 1. Agilent BioTek 406 FX washer dispenser.

Agilent BenchCel microplate handler

The Agilent BenchCel microplate handler is a compact microplate storage and automated microplate handling system designed for integration with many laboratory devices. The BenchCel microplate handler features a high-speed robot, and its modular design provides the flexibility and scalability required to meet the needs of the most diverse laboratory applications. The BenchCel can be powered by the Agilent VWorks automation control software or accessed through its ActiveX control for integration into any other software platform.

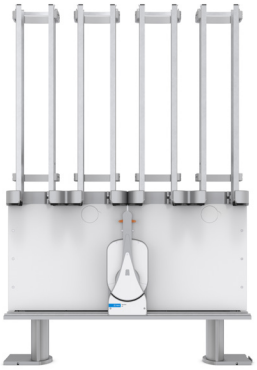

Figure 2. Agilent BenchCel microplate handler.

Agilent BioTek Synergy Neo2 hybrid multimode reader

The Agilent BioTek Synergy Neo2 hybrid multimode reader features proprietary Agilent BioTek Hybrid Technology, with independent optical paths that ensure excellent performance in all detection modes. Variable-bandwidth quad monochromators, sensitive high-transmission filter-based optics, laser TRF, and up to four PMTs provide ultrafast measurements.

Advanced environmental controls, including CO_2/O_2 control, incubation to 70 °C, and variable shaking, support live cell assays. The Agilent BioTek BioStack Neo delivers walk-away automation, high throughput, and barcode-labeled filter cubes to streamline workflows and limit errors. Powerful Agilent BioTek Gen6 data analysis software provides complete reader control, powerful data analysis, and LIMS integration.

Figure 3. Agilent BioTek Synergy Neo2 hybrid multimode microplate reader.

Materials and methods

Cell lines and plate seeding

Human lung carcinoma epithelial cell line (A549), fibrosarcoma cell line (HT-1080), and breast cancer cell line (MCF7) were cultured in Advanced-DMEM (A-DMEM) supplemented with 10% fetal bovine serum and penicillin-streptomycin at 37 °C in 5% CO_2. Cultures were routinely dissociated at 80% confluence. Cell lines were plated into Corning 3017 solid-white, 96-well microplates (Corning, New York, NY, USA) using A-DMEM with phenol red such that there were 20,000 total cells/well in 100 µL. After 24 hours to allow for attachment, the cytotoxic compounds were added to the cultures at various concentrations in 100 µL. After 48 hours of exposure, 150 µL of cell media was removed and diluted reagent added according to the outlines below.

Compound titrations

A 1:3 serial dilution of each compound was performed beginning with a 2x working concentration, resulting in an 11-point titration, including a zero-compound point. Compounds were added using a manual 12-channel pipettor to the 96-well plate in duplicate using a volume equal to the media volume in the wells, resulting in final concentration dilution series starting at 10 µM for camptothecin and doxorubicin or 50 µM for mitomycin C and oridonin.

Luminescent CellTiter-Glo assay

The plate was aspirated using the Agilent BioTek 406 FX washer dispenser 96-pin manifold before the addition of the CellTiter-Glo reagent, resulting in a 50 µL residual volume of media in each well. An equal volume of 50 µL per well of reconstituted CellTiter-Glo Cell Viability reagent (part number G7572; Promega Corporation, Madison, WI, USA) was added using the peripump dispenser fitted with a 1 µL dispense cassette. The plate was shaken on the 406 FX plate carrier with random orbital shaking for 2 minutes, then transferred to the Synergy Neo2 multimode reader. Luminescence was read using default settings for PMT gain (135) and the collection time (1 second).

Results and discussion

Dose-response curves were generated using a nonlinear, variable slope, four-parameter fit by plotting [compound] versus response. Differences in response to the various compounds of up to several orders of magnitude can be seen between the various cell lines tested, with the most pronounced being the minimal response seen when A549 cells were treated with oridonin up to a concentration of 50 µM (Figure 4).

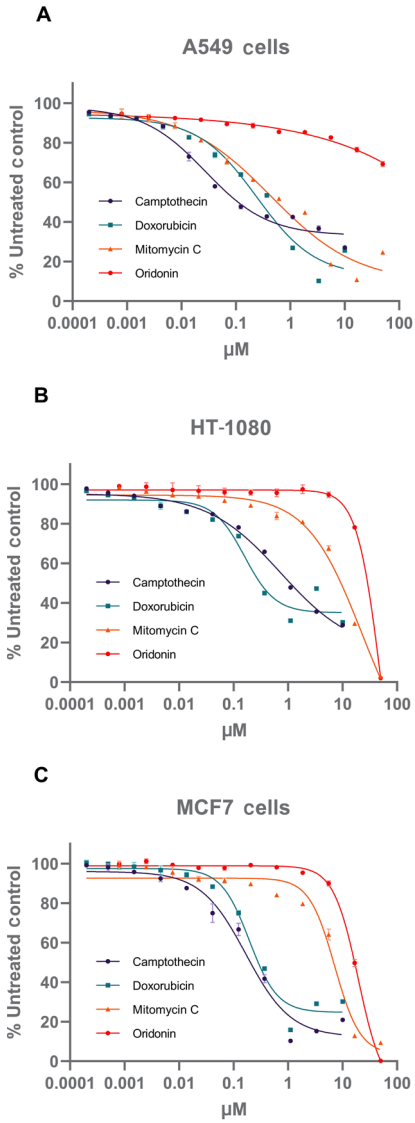

Figure 4. Dose-response comparison between cell lines. Dose-response curves represented as percent cell viability compared to untreated control for each cell line when subjected to an 11-point compound titration of the indicated compound: (A) A549 cells , (B) HT-1080 cells, and (C) MCF7 cells.

EC_{50} values determined from dose-response curves of the various compounds were tabulated for comparison (Table 1).

Table 1. EC_{50} values. The effect of various compounds on cell viability are depicted by determination of EC_{50} values. Note: The EC_{50} of oridonin was unable to be calculated against A549 cell as it showed little efficacy at concentrations up to 50 µM.

EC_{50} (µm)				
Cell Line	**Camptothecin**	**Doxorubicin**	**Mitomycin C**	**Oridonin**
A549	0.03	0.24	0.4	N/A
HT-1080	0.74	0.16	22.8	50.9
MCF7	0.17	0.19	7.2	19.5

Plating cells in a volume of 100 µL or more in a standard 96-well microplate provides sufficient nutrients for cell attachment and proliferation during overnight incubations before performing cell-based assays. Adding 100 µL of 2x working concentration of titrated compound with sufficient force provides adequate mixing to ensure equal distribution to the cell monolayer without additional plate shaking. The resulting well volume of 200 µL can be problematic when homogeneous assays require an equal amount of reagent be added, exceeding the well capacity in a 96-well microplate format. Automated removal of cell media resulting in a defined residual volume allows the assay to be performed in an efficient manner as well as cost savings by lowering the volume of reconstituted assay reagent required per well.

Accurate and precise reagent addition is critical for obtaining the best results with screening reactions that benefit from using automated methods. These results demonstrate that the 406 FX is capable of aiding in both media removal and reagent dispensing, providing increased throughput and time savings. When combined with the BenchCel microplate handler, further increases in throughput and time savings can be realized.

Conclusion

The automation of liquid and microplate handling, as well as microplate reading, has revolutionized the field of cell-based assays, particularly in the assessment of cell viability and cytotoxicity through luminescent readouts. This study has showcased the numerous advantages of automation in this context, including enhanced precision, increased throughput, reduced human error, and cost-effectiveness.

Using advanced instrumentation, such as the Agilent BioTek 406 FX washer dispenser, the Agilent BenchCel microplate handler, and the Agilent BioTek Synergy Neo2 hybrid multimode reader, researchers have been able to efficiently automate various steps of the cell viability assay. These instruments offer a high degree of flexibility and scalability, allowing for the integration of automation into diverse laboratory applications.

The results presented here demonstrate the successful automation of the luminescent CellTiter-Glo assay. The dose-response curves and EC_{50} values obtained provide valuable insights into the effects of different compounds on cell viability across multiple cell lines. Notably, the study highlights the potential limitations of certain compounds, such as oridonin, in specific cell lines.

Furthermore, the optimization of reagent handling, including precise media removal and reagent dispensing, has been achieved through automation, leading to increased efficiency and cost savings. The combination of the 406 FX washer dispenser and BenchCel microplate handler has shown the potential for further improvements in throughput and time savings, ultimately enhancing the productivity of cell-based assays.

In summary, the automation of liquid and microplate handling, along with microplate reading, represents a significant advancement in cell-based assays, offering researchers a powerful tool to conduct high-throughput screening, assess compound efficacy, and evaluate cytotoxicity with greater accuracy and efficiency. This technology holds promise for accelerating research and drug development in various fields of cell biology and pharmacology.

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