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Automation of Promega CellTiter-Glo 2.0 Cell Viability Assay using Biomek i7 Hybrid Automated Workstation

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Abstract

Determination of cell health is a part of many cell-based screening assays. It is used both as a final end-point measurement and a simple method for cell culture quality control. There are a multitude of different microplate-based assays that can be used to quantify living, viable cells or the number of dead and dying cells. One commercially available cell viability kit is Promega's CellTiter-Glo 2.0 assay. This is a relatively fast and straightforward method that is highly amenable to automation using liquid handlers, like the Biomek i7 Hybrid Automated Workstation. Here we show that the automated method allows quantification of viable cell count over a wide range of cell densities and can accurately quantify the potency of cytotoxic chemical agents, like digitonin. Additionally, even at relatively low cell densities the automated assay setup is robust enough to be amenable to high throughput screening in 384-well format (Z' = 0.80). In summary, automated CellTiter-Glo 2.0 assay plate handling by the Biomek i7 Hybrid Automated Workstation performs excellently, while increasing throughput, requiring less user hands-on time, and reducing the chance of user-introduced error as compared to the manual assay workflow.

Introduction

Determination of cell viability and toxicity are common end points in many cell-based screening paradigms, such as the identification of new chemotherapeutic agents or the discovery of new biologics via monitoring antibody-dependent cellular cytotoxicity. Additionally, monitoring viability of the cells within a microplate well can be a quality control step to allow data normalization in assays that measure other cellular events, such as reporter genes, second-messenger signaling, or protein trafficking. A large number of plate-reader compatible assays have been developed to monitor cell health, and these can largely be divided into those that quantify cell viability (i.e. the number of living cells) and those that track cytotoxicity (i.e. the number of dead cells). Cytotoxicity assays generally employ stains that selectively label dead cells (Trypan blue exclusion) or monitor cell integrity through detection of membrane leakage of cellular components from dying cells.[1] Viability assays often measure common markers of health that are critical in cellular metabolic functions, like the ability of mitochondria to reduce cell-permeable dyes (MTT absorbance assay) or synthesize ATP.[1] One commercially available kit to monitor cell viability through ATP detection is Promega's CellTiter-Glo 2.0 assay.[2]

Luciferase catalyzes the oxygenation of luciferin using ATP and molecular oxygen in the presence of divalent magnesium ions. This reaction generates oxyluciferin, which is bioluminescent and can easily be quantitated by measuring luminescence on standard multimode microplate readers (Figure 1). In the CellTiter-Glo 2.0 assay paradigm, all the necessary reagents for this reaction except for ATP are provided in a single convenient reaction mixture. Cells growing in microplate wells are diluted 1:1 with the kit provided CellTiter-Glo 2.0 reagent. The ATP that is required by the luciferase reaction is provided by lysing the living cells within the microplate well that is being measured. The amount of light that is generated by luciferase is proportional to the amount of ATP that is present, which is dependent upon the number of viable cells. CellTiter-Glo 2.0 has several notable improvements over previous iterations of the assay. The kit does not require combination of any reagents into a master mix and has prolonged stability at 4°C and ambient temperature. Further, the assay is homogenous, requiring no cell-media separation step and can be performed directly in culture wells.[2] This assay requires precise pipetting

and plate handling to obtain high quality results, making this workflow a candidate for laboratory automation with the use of liquid handling robots, such as the Biomek i7 Hybrid Automated Workstation (Figure 2).

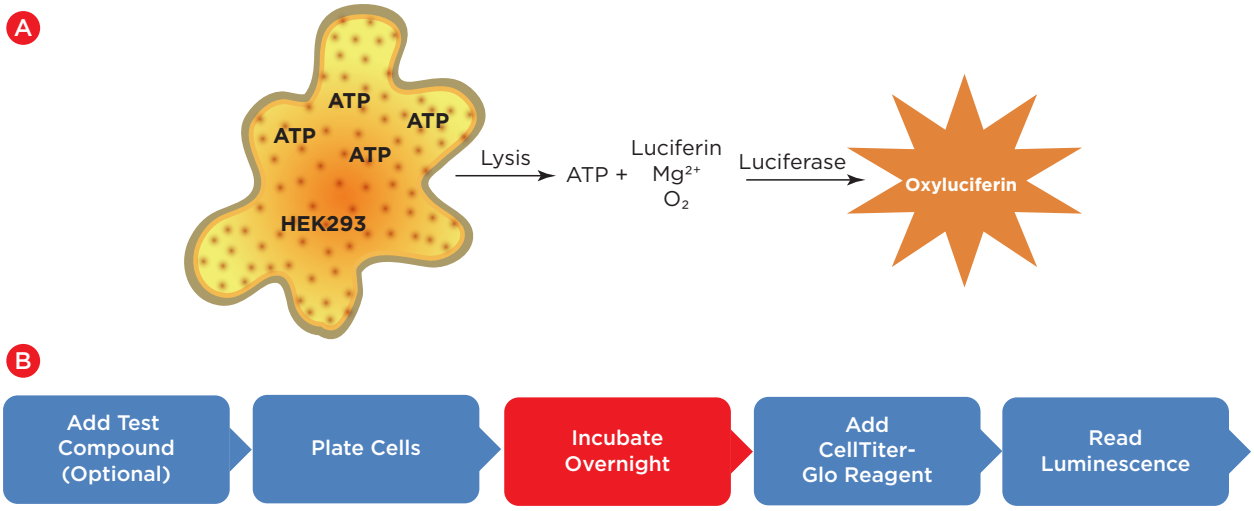

Figure 1. (A) Mechanism of ATP-dependent luciferase bioluminescence. (B) Workflow for Promega CellTiter-Glo 2.0. Steps that were performed offline of the Biomek i7 Hybrid Automated Workstation are highlighted in red and automated steps are indicated in blue.

The Biomek i7 Hybrid Automated Workstation is an automated liquid handler that is capable of efficiently performing the complex liquid handling steps of cell-based screening workflows. This minimizes the number of required user interactions and increases walk away time, freeing the operator to attend to other laboratory tasks. The multichannel pod can be equipped with a 96-well head that can accurately pipette 1 to 1200 µL or a 384-well head that is accurate over the range of 0.5 to 60 µL. Additionally, the 8-channel Span-8 pod is accurate from 1 to 1000 µL. The Biomek i7 Hybrid Automated Workstation supports 45 deck positions and can be directly fitted with orbital shakers, heating/cooling Peltiers, and tip-washers for plate and sample processing. Further, the Biomek i7 Hybrid Automated Workstation supports integration with other instruments, such as thermocyclers, automated incubators, barcode readers, plate washers, plate readers, centrifuges, and more, depending on user and workflow needs. Here we show that automated processing of the Promega CellTiter-Glo 2.0 Cell Viability Assay using a Biomek i7 Hybrid Automated Workstation provides excellent results. The automated workflow reduces the hands-on time and reduces the chances of user handling errors.

Figure 2. Biomek i7 Hybrid Automated Workstation

Methods

Cell Culture

Lenti-X 293T cells (Takara) were maintained in DMEM supplemented with 10% FBS and 1% antibiotic/antimycotic (Gibco) at 5% CO_2 and 37°C. One day prior to running CellTiter-Glo 2.0 assay, cells were washed with DPBS, harvested with trypsin, pelleted at 300 x g, and resuspended in growth media. Cells were counted and diluted to achieve desired cell density. For digitonin concentration-response and Z' experiments, 5,000 cells per well were used. Cells were plated in 384-well white, tissue-culture treated plates (Corning) in 25 µL total volume and incubated at 5% CO_2 and 37°C for 24 hours prior to performing the assay. For all experiments, cells were plated in alternating wells to minimize crosstalk between wells, and wells not containing cells were filled with 50 µL PBS to minimize evaporative effects.

Digitonin Treatment

For concentration response experiments, digitonin was serially diluted using the Span-8 pod and Serial Dilution function of the Biomek i7 Hybrid Automated Workstation. Following dilution, 5 µL of 5X concentrated digitonin was added to the assay plate using the Multichannel pod, and the plates were then spun at 1500 rpm in the on-deck microplate centrifuge (Agilent). This was followed by the addition of 20 µL of 293 cells diluted to 2.5 $x10^5$ cells/mL to achieve 5,000 cells per well. Plates were then spun at 1500 rpm and incubated at 5% CO_2 and 37°C for 24 hours.

Z' HTS Suitability

To negative control wells (5,000 cells, high viability), 5 µL of growth media was added using Span-8 pod, and to positive control wells (0 cells, low viability), 25 µL of growth media was added using Span-8 pod. This was followed by centrifugation at 1500 rpm in the on-deck microplate centrifuge (Agilent). Then, 20 µL of 293 cells diluted to 2.5 $x10^5$ cells/mL were added to negative control wells, and plates were then spun at 1500 rpm and incubated at 5% CO_2 and 37°C for 24 hours.

CellTiter-Glo 2.0 assay

Prior to performing assays, all required reagents and assay plates were incubated on the Biomek i7 Hybrid Automated Workstation deck for 15 minutes in the dark to allow for temperature equilibration. CellTiter-Glo 2.0 assays were then performed according to manufacturer recommendations. The deck layout for the automated Biomek method is shown in Figure 3. Following 15 min ambient temperature incubation, to each well containing cells (or media control), 25 µL of CellTiter-Glo 2.0 reagent was added using Multichannel pod and plate was spun at 1500 rpm using microplate centrifuge (Agilent). Assay plates were then subjected to orbital shaking at 300 rpm for 2 minutes, spun at 1500 rpm, and incubated in SpectraMax i3x (Molecular Devices) plate reader for 15 minutes. Following incubation, luminescence was measured using an integration time of 500 ms. Data was analyzed using Prism 8 software (GraphPad) and represents N=3 experiments, each performed with technical replicate wells.

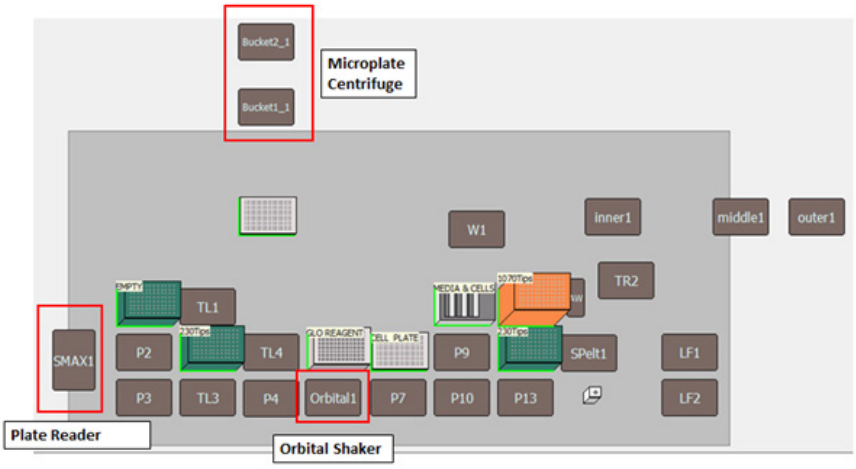

Figure 3. Deck layout for Biomek i7 Hybrid Automated Workstation Promega CellTiter-Glo 2.0 method. The automated method used the following components: one tip-loading ALP, six 1X1 ALPs, 1 trash bin, orbital shaker, Agilent Microplate Centrifuge, and microplate reader (Molecular Devices SpectraMax i3x).

Results

Promega CellTiter-Glo 2.0 is a fast and simple way to assess cell viability through luminescence following the release of ATP from lysed cells (Figure 1). Here we show the development of an automated method for this kit using a Biomek i7 Hybrid Automated Workstation. The Biomek was equipped with an orbital shaker, Agilent VSpin microplate centrifuge, and SpectraMax i3x plate reader, which allowed automated plate processing following initial deck set up (Figure 3). This included addition of luminescence reagents and movement of plates to and from the microplate centrifuge and plate reader. The first set of experiments was designed to determine the linear range of the CellTiter-Glo 2.0 assay kit by measuring luminescence at multiple cell densities ranging from 0 to 10,000 cells per well. Cells were serially diluted and plated in growth media using the Biomek i7 Hybrid Automated Workstation. Plates were then moved to a cell incubator overnight, and the next day the automated CellTiter-Glo 2.0 protocol was performed according to manufacturer recommendations. As can be seen in Figure 4, excellent linearity (R^2 = 0.99) was observed over HEK293 cell densities ranging from 173 to approximately 5,000 cells per well. At the highest cell densities, tested (6,700 and 10,000 cells per well) a loss of linearity was observed (data not shown), so these values were excluded from the analyses. This experiment showed that the Biomek-automated CellTiter-Glo 2.0 plate processing method could accurately quantitate viable cells over a broad linear range. Further these data were equivalent to results displayed in the manufacturer's product literature.[2]

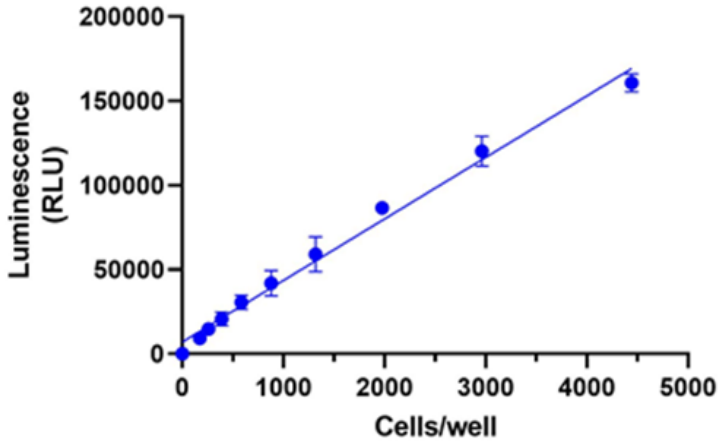

Figure 4. CellTiter-Glo luminescence activity from varying densities of LentiX-293 cells. Assay plates were prepared with Biomek i7 Hybrid Automated Workstation. R^2 = 0.99, data represents Mean ± S.D. of N=3 cell dilutions, each prepared with duplicate wells.

We next sought to show that the automated method could accurately measure concentration-dependent cytotoxicity. Digitonin was selected as a cytotoxic control compound, as it is often used as an agent for permeabilizing and solubilizing cell membranes.[3] A series of eight half-log serial dilutions of digitonin was prepared using the Serial Dilution step in the Biomek 5 software, and cells were treated with compound overnight at 37°C and 5% CO_2. Following incubation, the automated protocol was performed and cell viability was plotted as a function of digitonin concentration (Figure 5). A robust, concentration-dependent decrease in cell viability was observed, indicative of cytotoxicity (Figure 5). The IC_{50} value determined from this experiment was 11 µM, which agreed well with previous reports of digitonin toxicity in HEK293 cells.[4] This further showed the utility of the Biomek-automated CellTiter-Glo plate processing method, as accurate determination of cytotoxic compound potency could be measured.

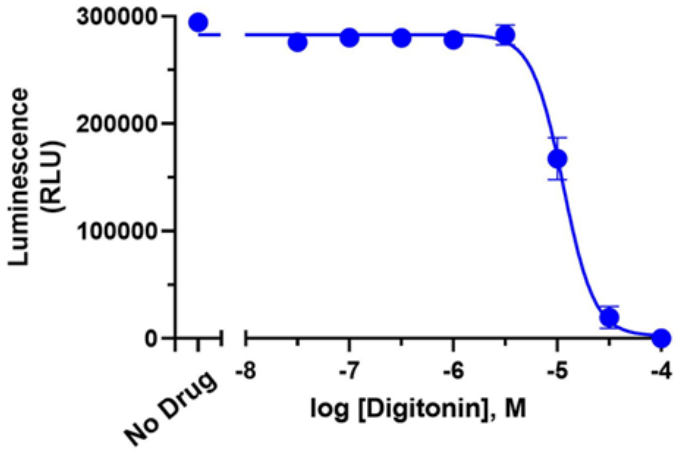

Figure 5. Digitonin concentration-dependent decrease in cell viability was measured using CellTiter-Glo 2.0 assay automated on Biomek i7 Hybrid Automated Workstation. IC_{50} = 11 µM, data represents Mean ± S.D. of N=3 digitonin dilutions, each prepared with duplicate wells.

In addition to measuring the number of viable cells in a well and the potency of cytotoxic chemicals like digitonin, the CellTiter-Glo 2.0 assay can also be used for high throughput screening campaigns to identify compounds that affect cell viability. One of the most important factors in the successful completion of any high throughput screening campaign is the identification of appropriate controls that display robust and reproducible results between wells. One way to determine the suitability of an assay for screening that has been widely adopted is the calculation of Z'.[5] This is a simple statistical parameter that takes into account the difference in the average values and the standard deviation of positive and negative controls. These values are reported in Table 1. For the purpose of the CellTiter-Glo 2.0 assay, 5,000 cells per well was selected as the negative control (high assay signal). Media alone (0 cells per well) was selected as a positive control, as this simulated a screening well that contained no viable cells due to cytotoxicity. Positive and negative control wells were plated with 24 replicates each, and the automated CellTiter-Glo 2.0 method developed above was performed (Figure 6). The Z' value obtained from this proof-of-concept experiment was 0.80, which was well above the accepted threshold of 0.5 for an assay that is excellently suited for screening. This indicated that the automated method results were robust enough to be considered for future screening efforts.

It is notable that automated cell incubators, like the Thermo Scientific CytoMat, can be integrated with the Biomek i7 Hybrid Automated Workstation. This would allow complete end-to-end automation of the CellTiter-Glo 2.0 workflow, reducing the hands on time. Additionally, the use of Beckman Coulter Data Acquisition and Reporting Tool (DART) software could be used to simplify data handling and plate tracking during the automated method.

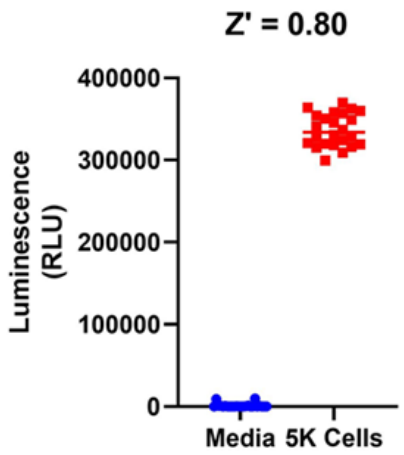

Figure 6. Suitability of automated CellTiter-Glo 2.0 workflow for high throughput screening. Individual values of positive (Blue) and negative (Red) control wells used for Z' calculations.

Cells per well	Control	Mean	S.D.	Z'
0	**Positive**	1,066	2,644	0.80
5000	**Negative**	336,602	19,609	

Table 1. Luminescence value statistics for control wells used to calculate Z'

Summary

Determination of cell viability is an important part of many cell-based assay workflows, as both a final endpoint and a simple method for cell culture quality control. Many commercial methods are available to measure viability and cytotoxicity, and one which has gained popularity is the Promega CellTiter-Glo 2.0 assay kit. This is a robust, fast and straightforward method that is highly amenable to automation using liquid handlers, like the Biomek i7 Hybrid Automated Workstation. Here we show that the automated Biomek method performs favorably compared with data presented in the manufacturer's technical bulletin[2] and previously published peer-reviewed reports.[4] We were able to successfully miniaturize the assay to 50 µL total volume in 384-well format, allowing for cost savings on reagents and increasing throughput. Additionally, control experiments showed that the Biomek automated protocol displayed excellent linearity over the range of 173 to approximately 5,000 cells per well. We also highlighted the utility of the automated method in determining the potency of the cytotoxic small molecule digitonin. Finally, we showed that the method could also be adapted for high throughput screening, as a Z' value of 0.80 was obtained for 5,000 HEK293 cells per well. Together the data presented here highlight a miniaturized and automated Promega CellTiter-Glo 2.0 workflow that reduces the hands-on time required by the user, freeing them to attend to other laboratory tasks, and reducing the chances of user handling errors.

References

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Materials

Equipment	Manufacturer
Biomek i7 Hybrid Automated Workstation	Beckman Coulter Life Sciences
Orbital Shaker ALP	Beckman Coulter Life Sciences
Microplate Centrifuge	Agilent
SpectraMax i3x	Molecular Devices

Table 2. Instruments used

Reagents	Manufacturer	Part Number
Lenti-X 293T cell line	Takara	632180
DMEM, high glucose pyruvate	Gibco	11-996-065
Antibiotic/Antimycotic 100X	Gibco	15-240-062
Fetal Bovine Serum	Gibco	16-000-044
DPBS	Gibco	14-190-144
Trypsin	Gibco	25-200-072
Digitonin	TCI Chemicals	D0540
CellTiter-Glo 2.0 Assay Kit	Promega	G9242

Table 3. Reagents used

Consumables	#	Manufacturer	Part Number
384-well plate, white, TC treated, barcoded	1	Corning	3570BC
Biomek 96-well microplate,	1	Beckman Coulter Life Sciences	609844
Biomek 96-deep well, square	1		609681
Biomek i-Series, 1070 µL pipette tip, sterile	1		B85884
Biomek i-Series, 230 µL pipette tip, sterile	2		B85906

Table 4. Consumables used per run