

APPLICATION NOTE

Improved cell viability for multi-day cell-based microplate reader assays

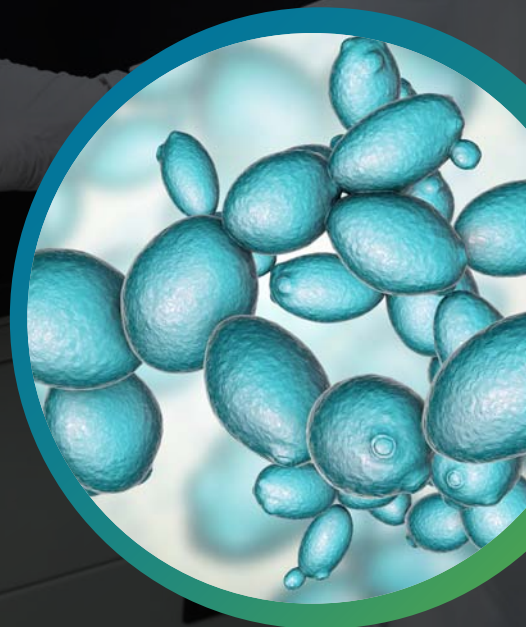
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Introduction

Live cell-based assays are performed routinely in pharmaceutical, environmental and toxicology laboratories to study cell growth, viability, cytotoxicity, and many other parameters. When assessing the response of cells to drug or compound treatment, it is often necessary to monitor cellular parameters over the course of several days to ascertain the most effective treatment window for a given cell or three-dimensional culture model. Consistent cell health is essential to maintain with these assays to ensure sensitivity and reliability of the data. Temperature and gas composition are key factors that should be controlled to ensure assay reproducibility. Maintaining healthy cells ideally involves mirroring the conditions of an incubator inside a microplate reader.

Most microplate readers today have a temperature control feature that enables maintenance of a constant 37°C during long cell-based experiments. Additionally equipping a reader with a gas mixer that can deliver a specified mix of gases, including above-ambient CO₂, can better match incubator conditions to maintain optimal cell health over extended times. Here we demonstrate the improvement in cell viability that is achieved with a SpectraMax® iD5e or iD3s Multi-Mode Microplate Reader with SpectraMax® aer

Gas Mixer, compared to a reader without a gas mixer. U-2 OS cells were grown in 96-well plates for 72 hours under different incubation conditions. Cell viability was measured by an endpoint luminescent ATP assay at 72 hours or monitored at intervals throughout the time course using the RealTime-Glo™ MT Cell Viability Assay.

Benefits

- Gas mixer enables fine tuning of the cell culture environment
- 3-day cell viability similar to that achieved with a standard cell culture incubator
- Straightforward user interface for easy gas mixer setup

Materials

- U-2 OS osteosarcoma cells (ATCC cat. #HTB-96)
- Growth medium for U-2 OS cells:
 - McCoy's 5A (Modified) Medium (Thermo Fisher cat. #16600082)
 - 10% fetal bovine serum (FBS, Avantor® Seradigm cat. #1500-500)
 - Penicillin/streptomycin (Thermo Fisher Scientific cat. #15070-063)
- RealTime-Glo MT Cell Viability Assay (Promega cat. #G9711)
- CellTiter-Glo 2.0 Cell Viability Assay (Promega cat. #G9241)
- 96-well black-walled, clear-bottom cell culture microplates (Corning cat. #3904 or 3603)
- ibiSeal self-adhesive cover film, 76.0 mm × 114.0 mm, sterilized (ibidi cat. #10874)

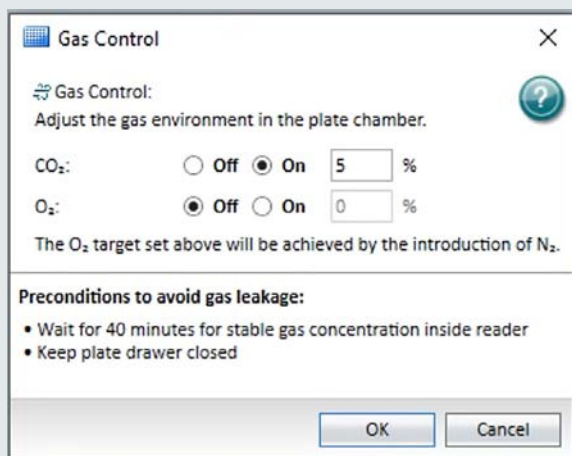


Figure 1. Gas control user interface in SoftMax Pro Software. CO₂ levels from 0.1% to 15.0%, and O₂ levels from 1.0% to 21.0%, can be set here.

Methods

- Cell plating: U-2 OS cells from one confluent T75 flask were trypsinized and suspended in 20 mL media, then counted. Cells were 90% viable, with 3.3×10^5 live cells/mL. Cells were diluted in media and seeded into wells of 96-well black/clear cell culture microplates at 4000, 2000, and 1000 cells/well, with 100 μ L per well and 18 wells per seeding density. Edge wells were filled with 200 μ L/well of PBS, and ibiSeals were applied, to minimize evaporation.
- Assay setup: To half of the wells containing cells, RealTime-Glo MT assay reagents were added, with a final well volume of 200 μ L. These wells were monitored at one-hour intervals over the 3-day incubation period.
- Incubation conditions: Plates were incubated in (1) a cell culture incubator at 37°C/5% CO₂, (2) a SpectraMax iD5e or iD3s Multi-Mode Microplate Reader with SpectraMax aer Gas Mixer, set to 37°C with 5% CO₂ (Figure 1), and (3) a SpectraMax reader set to 37°C with ambient CO₂ (no gas mixer).
- RealTime-Glo MT assay: Assay plates were read using the luminescence detection mode of the SpectraMax iD5e or SpectraMax iD3s reader. During the 3-day incubation in the reader, luminescence measurements were taken every hour. Growth curves were viewed as kinetic traces using SoftMax® Pro Software, and viability results were plotted by taking the maximum minus minimum signal for each well.
- CellTiter-Glo 2.0 assay: At the end of the 3-day incubation, CellTiter-Glo™ 2.0 (Promega) reagent was added to the wells, and the plates were shaken for 2 minutes, followed by incubation at room temperature for 10 minutes. Luminescence was then read on a SpectraMax iD5e or iD3s reader.
- Data analysis & graphing: Data generation was facilitated using preconfigured protocols in SoftMax Pro software. All data were analyzed and results plotted using SoftMax Pro software.

Results

Monitoring growth by measuring luminescence (RealTime-Glo MT) at 1-hour intervals resulted in the growth curves shown in Figure 2. Cells seeded at higher densities showed a reduction in growth rate during the third day of growth, whereas at the lowest density seeded, cells continued to grow at a more constant rate.

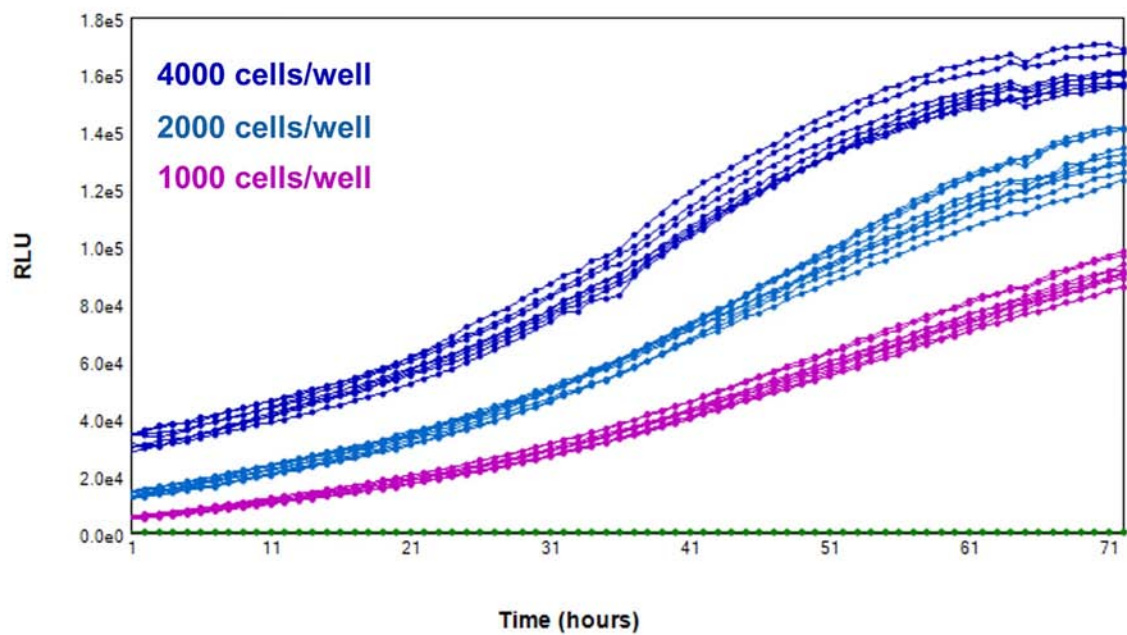


Figure 2. Growth monitored over the course of 3 days inside SpectraMax iD5e reader with SpectraMax aer gas mixer, using the RealTime-Glo MT assay.

As expected, cells assayed with a luminescent ATP assay showed higher signal at higher seeding densities after three days in culture. When compared to endpoint readings of the RealTime-Glo MT-assayed cells on day 3, the overall RLU magnitudes were higher with the ATP assay, but trends for RLU vs. cells seeded per well were similar, indicating the suitability of either assay for assessing viability (Figure 3).

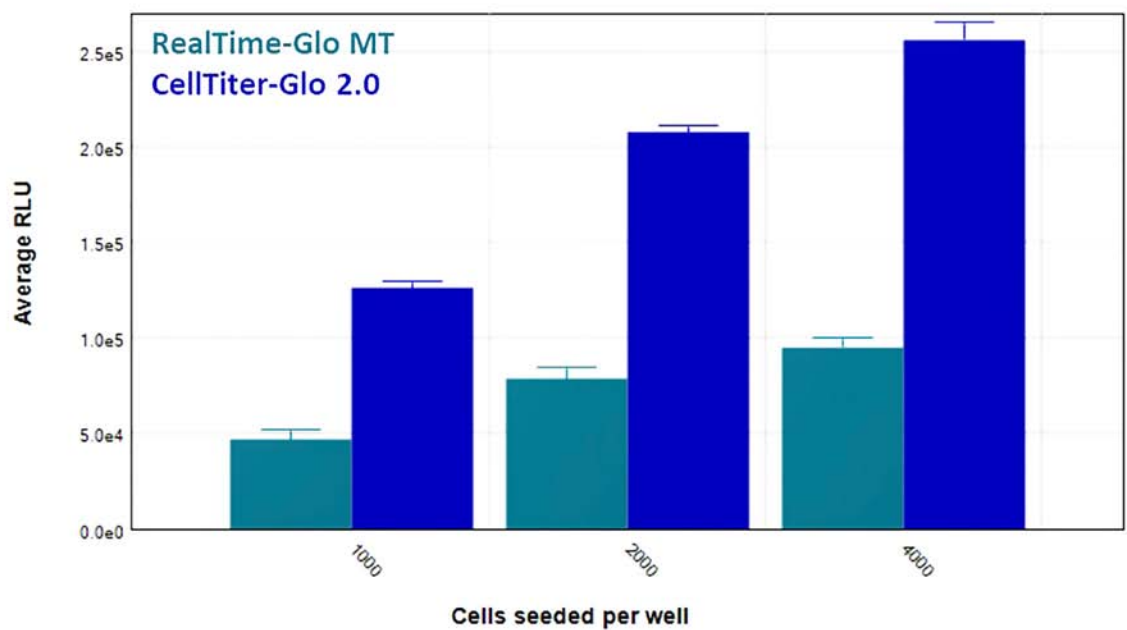


Figure 3. Cell viability after three days in a SpectraMax iD3s reader at 37°C/5% CO₂, with RealTime-Glo (light blue) and CellTiter-Glo (dark blue) readouts.

Results

Viability of cells incubated in the SpectraMax readers equipped with gas mixer fared better than those incubated in a plate reader without a gas mixer (Figure 4). The SpectraMax aer gas mixer set to deliver 5% CO₂ to the plate reader's read chamber enabled cell health more comparable to that observed when cells were kept in a standard cell culture incubator.

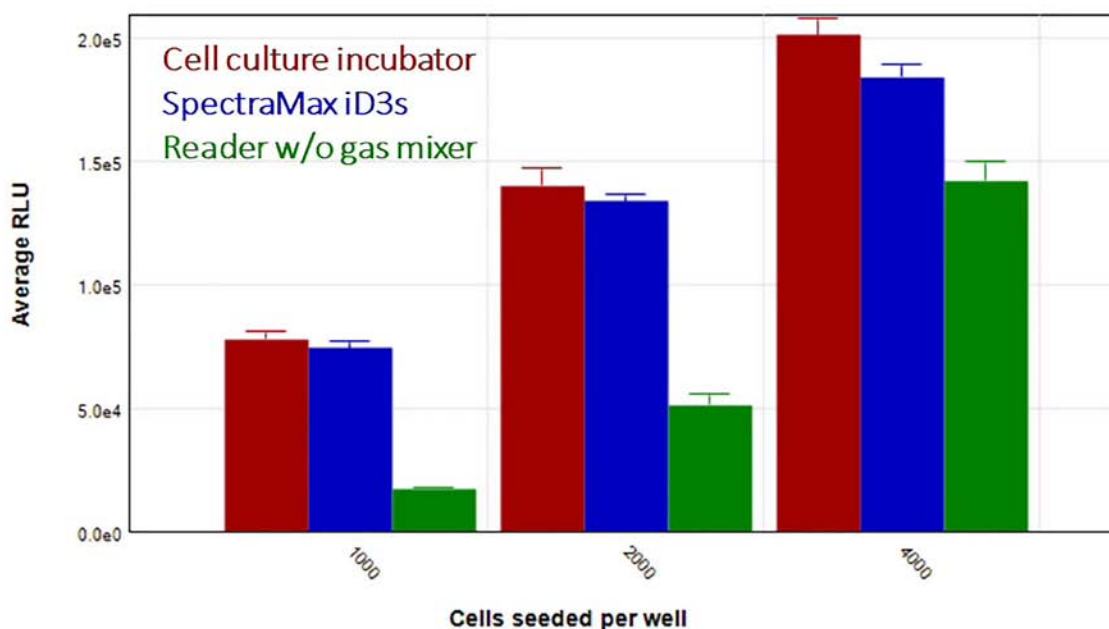


Figure 4. Comparison of cell viability after three days of incubation in a cell culture incubator (red), SpectraMax iD3s reader with SpectraMax aer gas mixer (blue), and a SpectraMax reader without a gas mixer (green). Data shown here were generated using the CellTiter-Glo assay.

Conclusion

SpectraMax iD5e and iD3s readers with SpectraMax aer gas mixer offer scientists environmental control options that allow for better cell viability and growth for assays where the detection of results is carried out for several hours or days. SoftMax Pro Software can be set up to generate measurements at regular intervals throughout multi-day experiments, ensuring reliable data collection without the need for inconvenient user intervention.

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