



Automation of Cell Viability Assays Utilizing Microfluidic Dispensing Technology

Paul Nguyen¹, Paul Ventura², Jeremy Lambert², Eric Veien², Vladislav Zarayskiy¹, Jonathan O'Connell¹

FORMULATRIX

¹FORMA Therapeutics, Watertown, MA ²Formulatrix, Inc., Bedford, MA



Introduction

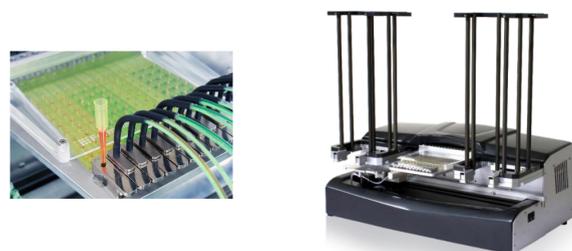
Cellular viability assays are widely used to investigate the potency or toxicity of biologically active molecules. One of the most widespread techniques to measure cellular viability is a luminescent CellTiter-Glo® (CTG) Assay which is a homogeneous method based on quantitation of the ATP present, an indicator of metabolically active cells. The "add-mix-measure" format results in generation of a luminescent signal proportional to the amount of ATP and, thus, proportional to the number of metabolically active cells present in culture. It is designed for use with multiwell formats, making it ideal for automated high-throughput screening, cell proliferation and cytotoxicity assays. However, in our hands, CTG has limitations for use on automatic platforms, due to the fact that the reliability of dispense degrades over extended run times, primarily as a result of liquid dispensers becoming clogged.

We have tested the use of CTG in two liquid dispensers: Multidrop® Combi Liquid Dispenser (ThermoFisher) and Tempest® (Formulatrix). The Multidrop consistently provided accurate and precise dispenses data during daily operations where many plates were dispensed in rapid succession. However, when runs had periods of inactivity (such as overnight, or longer pace-times), CTG would frequently clog the cartridge lines. Data obtained with Tempest has been found to be more reliable in extended run scenarios. Using the Tempest in a fully automated setting with recirculation turned on allows experiments been performed in multiple time-based formats, including overnight runs and longer pace-times. We have also demonstrated that the Tempest provides better precision in 1536-well plate format.

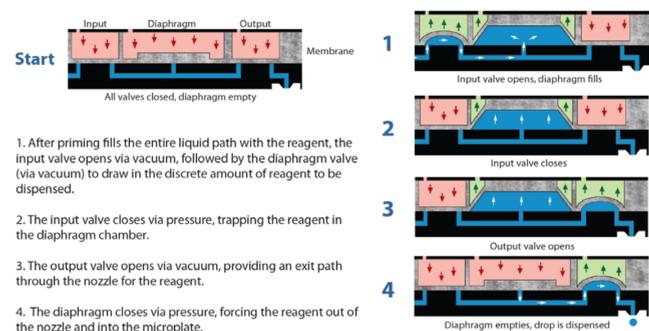
Materials & Methods

SNU-1 cells were cultured in suspension T-175 cm² flasks with RPMI media containing 10% FBS and 1% Pen/Strep. Cells were incubated in 5% CO₂ at 37°C and 98% relative humidity. To perform viability studies were cultured in 1536-well plates (Corning #3893) containing 5 ul media per well. Cells were incubated in 5% CO₂ at 37°C and 98% relative humidity for up to 72 hours. Media and cells were plated using either the Tempest from Formulatrix or Multidrop Combi Liquid Dispenser.

The Formulatrix Tempest is a non-contact, bulk reagent dispenser configurable to simultaneously deliver any volume of up to 12 separate reagents through 96 individually controlled nozzles. The Tempest's unique microfluidic valve cluster dispenses discrete volumes of liquid using positive displacement technology and is capable of accurate and precise dispenses to 96-well, 384-well and 1536-well plates. In addition, using a standard pipette tip as the source reservoir reduces the dead volume to 100 µL, optimal for precious sample and reagent dispenses.



The Tempest supports dispensing up to 12 different reagents to 96 wells simultaneously with volumes as low as 200 nL. The system is capable of returning any excess sample from the microfluidic chip back into the reagent reservoir. When using samples of limited quantity, a pipette tip can be used as reagent reservoir connected directly to the dispense head. Optional integrated stackers enable walk-away automation in a compact footprint.



Schematic of Tempest microfluidic dispensing technology. The instrument can be configured with up to 12 dispense chips, each chip with 8 independently controlled dispense nozzles. The geometry of the chip supports dispensing of cells and beads without clogging and CV less than 5%.

Metabolic activity of cells was measured using addition of CellTiterGlo (Promega, Madison, WI). Plates were imaged with Envision (PerkinElmer) and analyzed for metabolic activity with the ActivityBase, IDBS).

Results

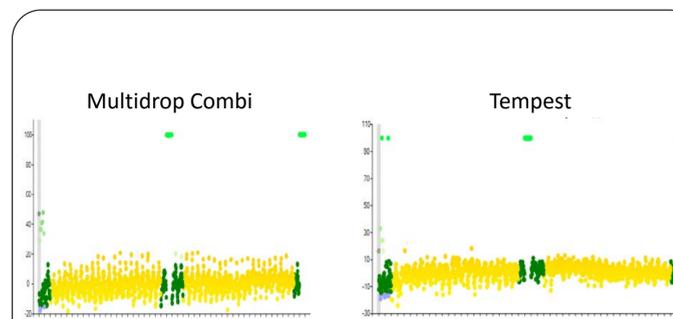


Fig.1 Cell dispensing precision assessed by CellTiterGlo addition at Time=0. 1 ul of CTG was added to 5 ul of media containing cells at time=0 (within an hour of adding cells to 1536-well plate (Corning 3893)). CTG dispensing with Tempest exhibited less variability and dispersion with cells (dark green and yellow circles) in all columns comparing to cells dispensed with Multidrop Combi.

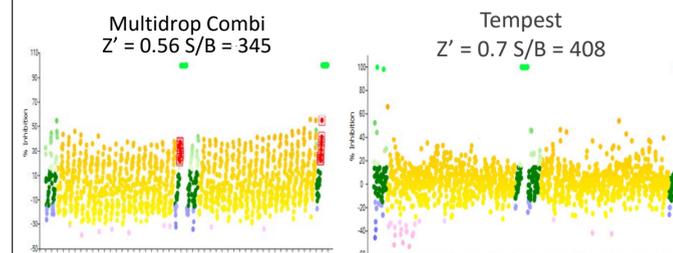


Fig.2 Cell viability assessed by CellTiterGlo addition at Time=72hr. 1 ul of CTG was added to 5 ul of media containing cells (after 72 hours of incubation in 5% CO₂ at 37°C). S/B was comparable between two methods, which means that cells on a plate were at a same level of metabolic activity. However, there was significantly less dispersion of data on a plate where CTG was dispensed with Tempest comparing to a plate where CTG was added with Multidrop Combi.

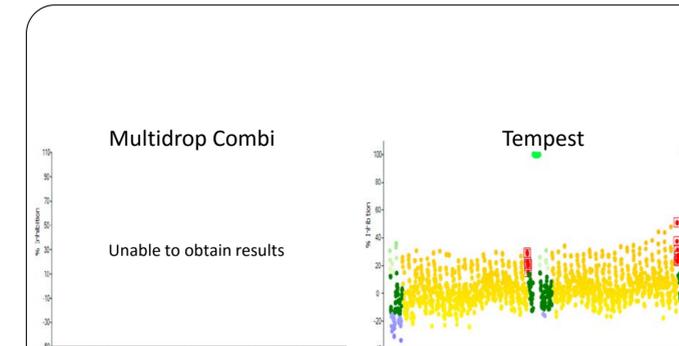


Fig.3 Assessment of cell viability after long period of inactivity. 1 ul of CTG was added to 5 ul of media containing cells (after 72 hours of incubation in 5% CO₂ at 37°C). CTG was left in the system for 16 hours (overnight). Data could not be generated with Multidrop Combi because the reagent clogs the cartridge lines after a long period of inactivity. Using Tempest in automated setting with recirculation turned on allowed us to avoid clogging issue and generate data after overnight incubation with a viscous reagent.

Conclusions

The purpose of this study was to assess the use of an automated liquid dispensing system with a plate-based reader for long run and long pace-time cellular viability studies. Very often cell viability readout should occur at the time after long inactivity or should be followed after prolonged inactivity. While using such viscous reagent as Cell Titer Glo we have found that in these situations Multidrop Combi Liquid Dispenser (ThermoFisher) cartridge lines are clogged, sometimes beyond repair. Utilizing the Tempest (Formulatrix) with recirculation feature turned on helps researchers to avoid clogging and perform CTG experiments with a high degree of precision and in a truly automated and time-independent manner. Overall, in our hands, Tempest exhibited higher degree of precision and lower dispersion of values in Cell Titer Glo addition, comparing to Multidrop Combi Liquid Dispenser.