Corning® Lambda™ EliteMax Semi-automated Benchtop Pipettor for High Precision Liquid Handling with Spheroids

Application Note

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Hilary Sherman and Ann Ferrie
Corning Incorporated, Life Sciences
Kennebunk, ME USA

Introduction

Consistent and reproducible pipetting is one of the foundations of any cell-based assay. Inconsistencies and errors from manual pipetting can lead to loss of time, samples, and ultimately results in poor quality data. In order to address these issues, automated systems are often employed as a solution, but they can be large and bulky, making them unsuitable for use in a biosafety cabinet. Add in the high cost of most of these systems and it is not surprising that these automated systems have not become a staple in every lab.

The Corning Lambda EliteMax Semi-automated Benchtop Pipettor aims to solve these problems by being compact enough to easily fit in a standard biosafety cabinet and cost-efficient enough for smaller research labs looking to automate their liquid handling. Designed for semi-automated pipetting of 96- and 384-well formats, the Corning Lambda EliteMax Pipettor comes with an integrated touchscreen display that allows for easy creation and implementation of protocols. Five deck positions, along with the added ability to pipet in rows or columns, allow for increased complexity of experiments. Here we demonstrate use of the Corning Lambda EliteMax Pipettor for a basic spheroid viability assay using dispensing, aspirating, and mixing functions. Our results demonstrate that the Corning Lambda EliteMax Pipettor can successfully seed, media exchange and assay 96-well spheroid microplates.

Materials and Methods

Spheroid Seeding

HT29 (ATCC® HTB-38) cells were cultured in Dulbecco's Modification of Eagle's Medium (DMEM; Corning 10-013-CM) containing 10% fetal bovine serum (FBS; Corning 35-010-CV). Cells were harvested with trypsin (Corning 25-052-CV) from a T-225 flask (Corning 431082), and resuspended at a concentration of 10,000 cells/mL. The Corning Lambda EliteMax Semi-automated Benchtop Pipettor (Corning 6070) was used to seed five 96-well spheroid microplates (Corning 4520) via landscape orientation at volume of 100 µL per well. A plate filling protocol was designed with 3 mix steps using an Axygen® single well reagent reservoir (Corning RES-SW96-HP-SI) prior to dispensing with Axygen sterile 96-well tips (Corning FX-250-R-S). Microplates were incubated at 37°C in a humidified CO_2 incubator for approximately 16 hours to allow for spheroid formation.

Medium Exchange

After overnight incubation, a plate-to-plate transfer protocol for the Corning Lambda EliteMax Pipettor was used to remove 60 µL of medium from each well of the spheroid microplates using the slowest setting. A plate filling protocol was subsequently used to add 60 µL of fresh medium, and plates were returned to the incubator for approximately 24 hours.

Assay

Approximately 48 hours after seeding, a plate-to-plate transfer protocol was initiated to remove 50 µL of medium followed by an addition of 50 µL of CellTiter-Glo® 3D Reagent (Promega G9681) using the plate filling protocol. Plates were shaken for 5 minutes per Promega's protocol, and then incubated at room temperature for an additional 25 minutes. Finally, plates were read for luminescence using the EnVision® multimode plate reader (PerkinElmer 2105-0010).

Results and Discussion

Automating the liquid handling of spheroid assays can be challenging due to the inherent risk of damaging or aspirating spheroids that are not attached to a surface. Controlling the height and speed of reagent manipulation are key parameters in ensuring the integrity of each sample. Using the Corning Lambda EliteMax Pipettor software easily allows the user to control the dispense/aspiration rate, height, and volume while operating, thus preventing disruption of the spheroids in culture. Our data show spheroid liquid handling with the Corning Lambda EliteMax Pipettor resulted in consistent luminescent signal across each plate and between plates, demonstrating consistent cell numbers within each well (Figure 1). Additionally, with CV values under 13% (Figure 2) and Z' values above 0.6 (Figure 3), demonstrating the robustness of the assay.

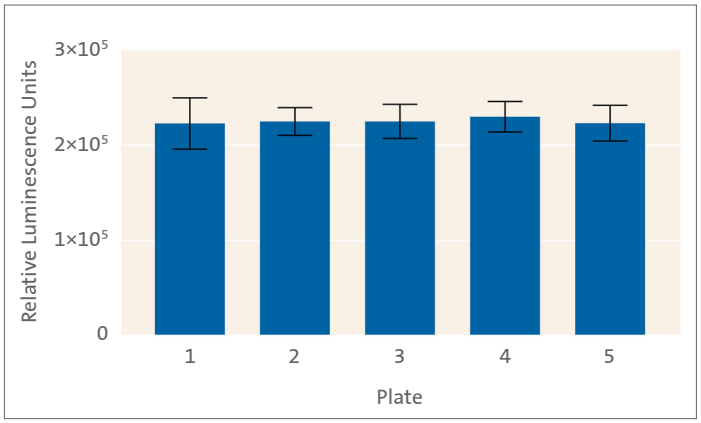

Figure 1. Consistent signal within and across spheroid microplates. Average luminescent signal generated from each of 5 plates containing HT29 spheroids. Data is average of 96 wells per plate shown with standard deviations.

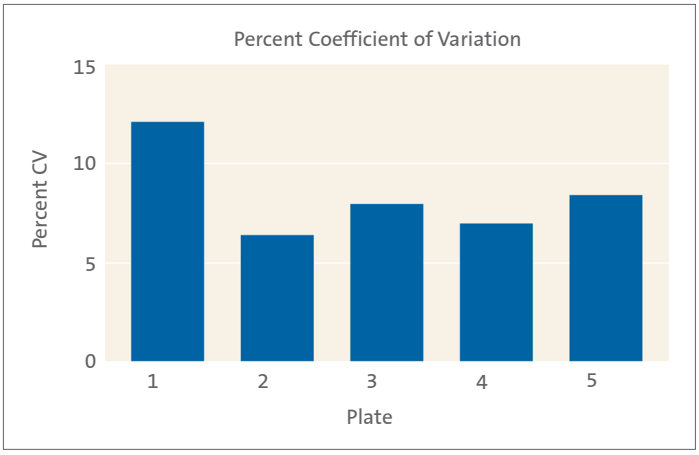

Figure 2. Low plate CV values. Percent CV values less than 13% from all 5 plates.

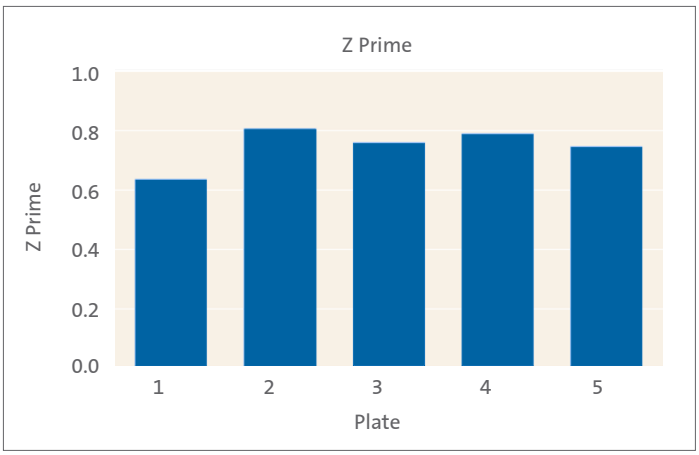

Figure 3. Excellent Z' values. Z' values above 0.6 for each of 5 plates tested.

Conclusions

To automate spheroid assays, proper tools are necessary to achieve desirable results. The Corning® Lambda™ EliteMax Semi-automated Benchtop Pipettor has the flexibility of larger more expensive systems with 5 deck positions, the ability to pipette in portrait or landscape orientation, while still being small enough to fit in a standard biosafety cabinet. Additionally, the touch screen display and user-friendly interface make the Corning Lambda EliteMax Semi-automated Benchtop Pipettor an ideal option for a benchtop liquid handler.

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Corning Incorporated
Life Sciences

www.corning.com/lifesciences

NORTH AMERICA
t 800.492.1110
t 978.442.2200

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Australia/New Zealand
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Chinese Mainland
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India
t 91 124 4604000
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t 81 3-3586 1996
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t 886 2-2716-0338

EUROPE
CSEurope@corning.com
France
t 0800 916 882
Germany
t 0800 101 1153
The Netherlands
t 020 655 79 28
United Kingdom
t 0800 376 8660

All Other European Countries
t +31 (0) 206 59 60 51

LATIN AMERICA
grupoLA@corning.com
Brazil
t 55 (11) 3089-7400
Mexico
t (52-81) 8158-8400