# Corning® Lambda™ EliteMax Semi-automated Benchtop Pipettor for Drug Screening Workflows

## Application Note

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## Introduction

Drug discovery is a costly and time-consuming process which starts with target identification and assay development with the hope of finding a candidate to take to market[1]. To identify potential candidates, screening campaigns are executed that often involve extensive libraries of compounds resulting in a scattering of compounds eliciting a response in a specific assay. These active compounds, often referred to as hits, undergo additional validations to confirm specificity and/or dose dependency. Use of an automated liquid handling systems are routinely employed to ease the burden of these processes, but are often complicated, bulky, and costly to use. The Corning Lambda EliteMax semi-automated benchtop pipettor, on the other hand, is easy to operate, compact enough to easily fit in a standard biosafety cabinet, and cost efficient enough for smaller research labs looking to automate their liquid handling processes. Designed for semi-automated pipetting of 96-well formats, the EliteMax benchtop pipettor can be used to seed cells, screen, cherry-pick hits, and serially dilute compounds for assessing dose dependency. Here, we demonstrate how the Corning Lambda EliteMax benchtop pipettor can be utilized to run an entire drug screening workflow utilizing A549 cells as a model.

## Materials and Methods

### Cell Seeding

A549 (ATCC® CCL-185) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Corning 10-013-CM) containing 10% fetal bovine serum (FBS; Corning 35-010-CV). Cells were harvested with 0.05% Trypsin/0.53 mM EDTA solution (Corning 25-052-CV) from a T-225 flask (Corning 431082) and resuspended at a concentration of 500,000 cells/mL in complete media.

The EliteMax benchtop pipettor was decontaminated and placed into a biosafety cabinet (BSC) prior to use in seeding 96-well flat bottom microplates (Corning 3903), via landscape orientation. 50 µL was dispensed into each well using a plate-to-plate transfer protocol programmed with 3 mix steps to ensure the homogeneity of the cells being aliquoted. A sterile Axygen® multiple well reagent reservoir with 8-channel trough (Corning RES-MW8-HP-SI) and Axygen sterile 96-well tips (Corning FX-250-R-S) were also placed on the deck. Microplates were incubated at 37°C in a humidified $CO_2$ incubator for 16 to 20 hours to allow for cell attachment prior to compound addition.

### Compound Screening

Compounds were diluted by adding 58 µL of media into 2 µL of an in-house compound library and mixed three times, using the plate-to-plate transfer protocol in conjunction with the Axygen multiple well reagent reservoir. The library consisted of compounds that were resuspended at a concentration of 10 mM in dimethyl sulfoxide (DMSO) or 100% DMSO as a control. 50 µL of diluted compound, or DMSO control, was then added to each well of a seeded microplate using the plate-to plate transfer protocol at a medium speed setting for dispense resulting in a final concentration of 166.7 µM of each compound. Microplates were then incubated at 37°C in a humidified $CO_2$ incubator for 16 to 20 hours prior to running a cell viability assay.

### Cell Viability Assay

On the third day, a plate-to-plate transfer protocol was used to remove 50 µL of media from each well of the 96-well microplate and discarded into a waste reservoir. Next, a plate-to-plate transfer protocol was used to add 50 µL of CellTiter-Glo® reagent, which was prepared according to manufacturer's protocol from the CellTiter-Glo luminescent cell viability assay kit (Promega G7572), to each well using a multiple well reagent reservoir. Cells were incubated at room temperature for 30 minutes prior to being measured for luminescence, via the PerkinElmer Envision® multimode plate reader (PerkinElmer 2105-0010), to assess the viability of cells in each well.

### Cherry-picking and Dilution Assay

To further assess compounds identified as hits from the screening, a cherry-picking protocol was written to select 4 compounds from the library plate. Selected compounds were individually transferred from the library plate to an Axygen 96-well clear V-bottom 600 µL PP storage plate (Corning P-DW-500-C-S) in triplicate. The protocol was designed to aliquot 4 µL from the 10 mM compound library plate and, in triplicate, transfer to row A of the storage plate. Next, a plate-to-plate transfer protocol was used to add 116 µL of media to compounds in row A from an Axygen multiple well reagent reservoir to attain a starting concentration of 166.7 µM. Using the same protocol, 100 µL was transferred, from a reagent reservoir containing media with 3.3% DMSO, to rows B-H. A dilution protocol was then used to serially dilute compounds 1:3 from row A to row G by transferring 50 µL from one row to the next mixing 3 times before transferring to the next row resulting a final concentration of 0.22 µM in row G.

No compound was added to row H which served as DMSO control wells. A plate-to-plate protocol was then used to transfer 50 µL of diluted compound or DMSO control to a microplate of A549 cells seeded as previously described. Compounds were incubated with cells for 16 to 20 hours and assayed for cell viability using the aforementioned cell viability assay protocol.

## Results and Discussion

Automating the drug screening workflow is essential for any drug discovery campaign. Here, proof-of-concept was provided for an automated compound screen involving cell seeding, preparing the compound screen plate, cherry-picking, serial dilution plate preparation, and performing a cell assay using the Corning® Lambda™ EliteMax benchtop pipettor for all liquid handling steps. Figure 1 shows the results from a 64-compound library screen with each blue dot representing the relative luminescent signal from a single compound using the CellTiter-Glo® luminescent cell viability assay kit. The gray line shows the average luminescent signal from DMSO control wells, which should have the highest luminescent signal associated with the highest cell viability, whereas the red line indicates 5σ below the DMSO control response indicating those compounds were considered hits by eliciting a strong decrease in cell viability.

Four of the 17 compounds were chosen for further analysis due to their known antiproliferative effects on A549 cells. Using the EliteMax benchtop pipettor cherry-picking capabilities, hit compounds were transferred from the source plate to a screening plate. Serial dilutions were subsequently performed to verify if the compound generated a dose-dependent response. Again, the EliteMax benchtop pipettor was used for the entire process from seeding cells, cherry-picking hits, serial dilutions, compound additions, and finally in performing a cell viability assay.

The average of 3 independent assays is shown in Figure 2 with standard deviations. TC50 values were determined to be 282, 23, 9, and 47 µm for compounds 1-4, respectively, demonstrating compound 3 has the highest potency while compound 1 has the lowest.

## Conclusions

To automate cell-based assays, proper tools are necessary to achieve consistent and reliable results. The Corning Lambda EliteMax benchtop pipettor has the flexibility of larger more expensive systems with 5 deck positions and the ability to pipette in portrait or landscape orientation, while still being small enough to fit in a standard biosafety cabinet. Additionally, the touchscreen display, ability to store hundreds of protocols, and user-friendly interface make the Corning Lambda EliteMax benchtop pipettor an ideal option for executing complete drug screening workflows.

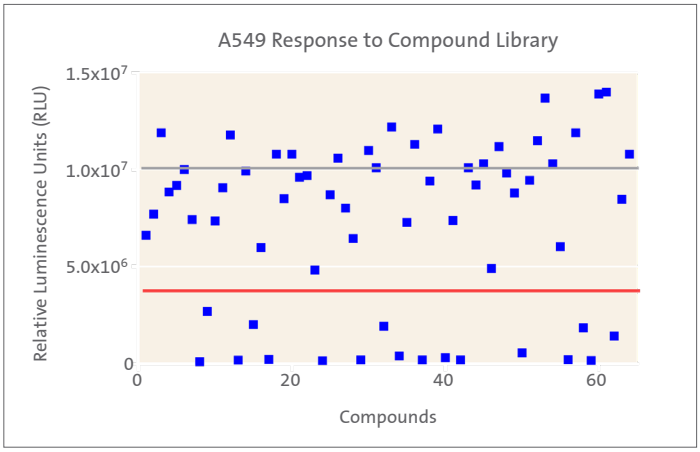


**Figure 1. Screening results after A549 cell exposure to compound library.** CellTiter-Glo was used to assess the impact of various compounds to A549 cells. Higher RLU indicating low or no impact on cell viability while lower RLU indicates the compound impacted cell health and viability. The blue dots represent all 64 compounds tested. The gray line is the average buffer response. The red line is 5σ below buffer response and was used to help determine which compounds should be analyzed further.

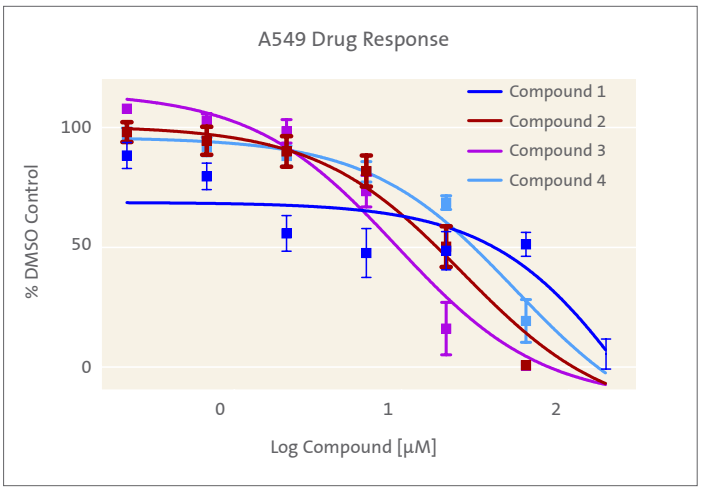


**Figure 2. Average dose response of A549 cells to cherry-picked compounds.** Relative luminescence units from the Corning Lambda EliteMax benchtop pipettor cherry-picked and serially diluted compounds as a percentage of DMSO control response. Data shown is the average of 3 independent assays with standard deviations. N = 9 per drug concentration resulting in TC50 = 282, 23, 9, and 47 µm for compounds 1-4, respectively as determined by nonlinear regression fit.

## References

1. Hughes JP, et al. Principles of early drug discovery. Br. J. Pharmacol. 162.6 (2011): 1239-1249.

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