



BIOSPOT® Workstation: Elevating Cell-Based Toxicity Assays with Nanoliter Precision

Authors: Sonja Tischlik¹, Julia Stifel¹, Thomas Clapes¹

¹Hamilton Freiburg GmbH, Georges-Köhler-Allee 302, 79110 Freiburg im Breisgau, Germany

Fully automated drug screening for parallel compound testing, ensuring low DMSO concentrations and precise, reproducible results.

- **Parallel automation and flexibility:**
Automated dilution series for eight compounds in one run, with adjustable concentrations and dispensing layouts.
- **Precision and low DMSO usage:**
Accurate nanoliter dispensing minimizes DMSO concentration, ensuring optimal conditions for cell-based assays.
- **Fully automated solution:**
Supports up to six plates, with automated aspiration, dispensing, and washing for increased efficiency and walk-away time.

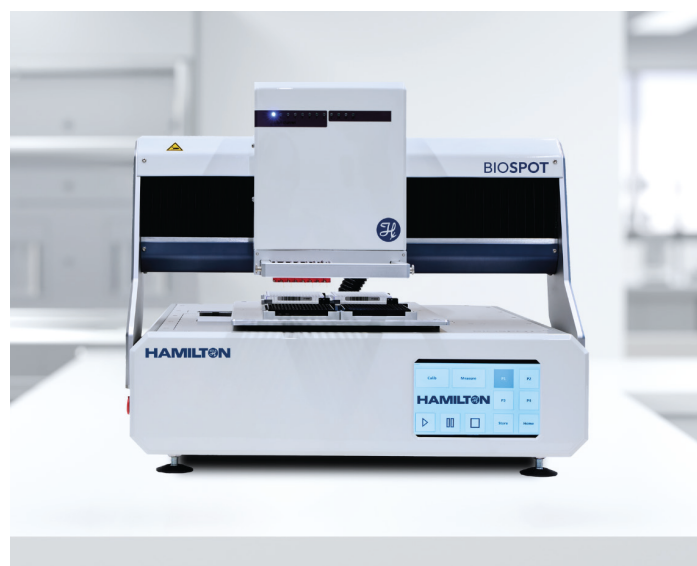


Figure 1: BIOSPOT® workstation.

The BIOSPOT® workstation simplifies the creation of compound concentration curves with precise nanoliter dispensing. This technology enables achieving the lowest possible compound concentrations while maintaining minimal DMSO levels.

Introduction

During drug development, initial hit compounds are evaluated in dose-response assays to determine their toxicity and efficacy. These experiments are fundamental in pharmacology and toxicology for elucidating the relationship between the dose of a compound and the biological response. Dose-response curves provide a quantitative basis for comparing substances, facilitating the determination of critical parameters such as EC_{50} and IC_{50} values, which are essential for assessing their clinical and therapeutic potential. Consequently, this approach plays a pivotal role in identifying promising candidates for preclinical and clinical development.^[1]

Typically, a dose-response curve is generated by applying a range of compound concentrations to a selected cell model. For toxicity assays, compounds are commonly diluted in DMSO and dispensed at 7 to 11 different concentrations onto cells. Following an appropriate exposure period, the compound's effect on cell viability is quantified using various live or death markers, often through fluorescence-based readouts that measure the intensity of signals correlated with living or dead cells.



Figure 2: PIPEJET® modules.

The eight PIPEJET® modules are shown with the pipes (yellow) inside the pipe guides (black), connected to the liquid reservoirs.

The precise nanoliter dispensing capabilities of the BIOSPOT® workstation enable the automated preparation of accurate concentration curves. By dispensing nanoliter volumes, the system ensures precise delivery of small compound quantities while minimizing DMSO content, which reduces potential cytotoxic effects and preserves assay integrity. Additionally, the 8-channel configuration of the BIOSPOT® workstation allows up to eight compounds to be tested simultaneously in a fully parallel mode, significantly enhancing throughput and reproducibility. Moreover, with its fully automated workflow, including integrated aspiration and dispensing, the BIOSPOT® workstation enhances efficiency and walk-away time, streamlining the generation of high-quality dose-response data.

System Description

BIOSPOT® workstation

The BIOSPOT® Workstation (Figure 1) is equipped with 8 PIPEJET® modules (Figure 2), utilizing innovative, non-contact piezo-actuated dispensing technology and a tray with capacity for up to 6 microtiter plates.

PIPEJET®

Each PIPEJET® (Figure 2) can independently aspirate liquid from any plate on-deck and precisely dispense nanoliter volumes.

SMARTDROP®

The integrated SMARTDROP® technology employs a fast shutter time camera to capture the drop mid-flight, ensuring precise volume calibration across various liquid types (Figure 3). This allows for immediate adjustments and quality control, delivering droplets ranging from 7 to 15 nanoliters with a resolution of 0.1 nanoliters.

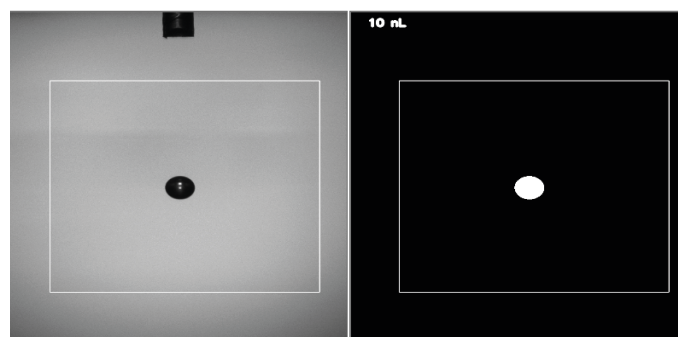


Figure 3: SMARTDROP® technology.

The SMARTDROP® camera captures the drop mid-flight to ensure precise volume calibration across various liquid types.

Workflow

Method

Dose-response curves were generated by a fully automated workflow, dispensing 8 known toxic compounds onto HEK293 cells and fluorescent readout of viability levels. The general workflow is depicted in Figure 4.

Choice of compounds and concentration range

The BIOSPOT® was used to create concentration curves of the following 8 compounds:

1. **Camptothecin:** inhibits DNA topoisomerase I, inducing DNA-strand breaks and apoptosis^[2]
2. **Digitonin:** permeabilizes cell membranes^[3]
3. **Doxorubicin:** inhibits DNA topoisomerase II, thus stopping DNA replication^[4]
4. **Etoposide:** inhibits DNA topoisomerase II, inducing cell cycle arrest, apoptosis and autophagy^[5]
5. **Nocodazole:** binds to tubulin and disrupts microtubule assembly/disassembly dynamics, preventing mitosis and inducing apoptosis^[6]
6. **Paclitaxel:** stabilizes tubulin polymerization, inducing mitotic arrest and apoptosis^[7]
7. **Terfenadine:** histamine H1-receptor antagonist, induces apoptosis^[8]
8. **Staurosporine:** protein kinase inhibitor and phosphatase kinase inhibitor^[9]

All tested compounds are described in the literature with distinct EC₅₀ values, simulating a scenario where the efficacy of a novel drug compound is unknown. A broad concentration range, from 100 pM to 50 µM, was selected to ensure optimal sensitivity for both low-potency and high-potency compounds. This range can be easily adjusted by modifying either the concentrations of the pre-dilutions or the drop volumes and numbers used during dispensing, providing flexibility for a wide array of compounds.

Experiment preparation

HEK293 cells were seeded into a 384 well plate (target plate) with a density of 10 000 cells/well in 50 µL/well cell culture media. Incubation for 24 h at 37 °C, 5 % CO₂ allowed them to settle and reach a confluency of 40 % before compound addition.

Parallelized generation of dose-response curves

Starting from a 10 mM stock solution, three pre-dilutions (0.5mM, 10µM, 50nM for Staurosporine, Paclitaxel and Terfenadine; 5 mM, 100 µM, and 500 nM for all other toxins) were prepared in DMSO in a 384-well microtiter plate (source plate). Both the stock solution and pre-dilutions were aspirated and dispensed fully parallelized into the corresponding wells using precise 10 nL drop volumes. The individual PIPEJET® modules of the BIOSPOT® workstation were calibrated with DMSO

to a final volume of 10 nL per drop using the SMARTDROP® technology.

Concentration curves for the 8 different compounds were generated in 4 replicates on a 384 well plate (target plate) according to the pipetting scheme in Figure 5.

DMSO backfill

To maintain consistency and avoid variability in assay conditions, all wells were backfilled with DMSO to a final DMSO concentration of 0.5 % per well. This step ensures uniform solvent levels across the plate as fluctuations in DMSO concentration could otherwise influence compound activity and assay results.

All dispensing steps were performed with a 200 µm dispense nozzle, allowing to dispense drops with an adjustable volume in the range between 7 nL and 15 nL. Silicon tubing was used to ensure material compatibility with DMSO.

Pipe washing

To prevent any cross-contamination or carryover between the solutions with different compound concentrations (stock and predilutions), thorough washing was performed fully automated before aspirating new liquid. The washing procedure involved three cycles with

Visual Workflow

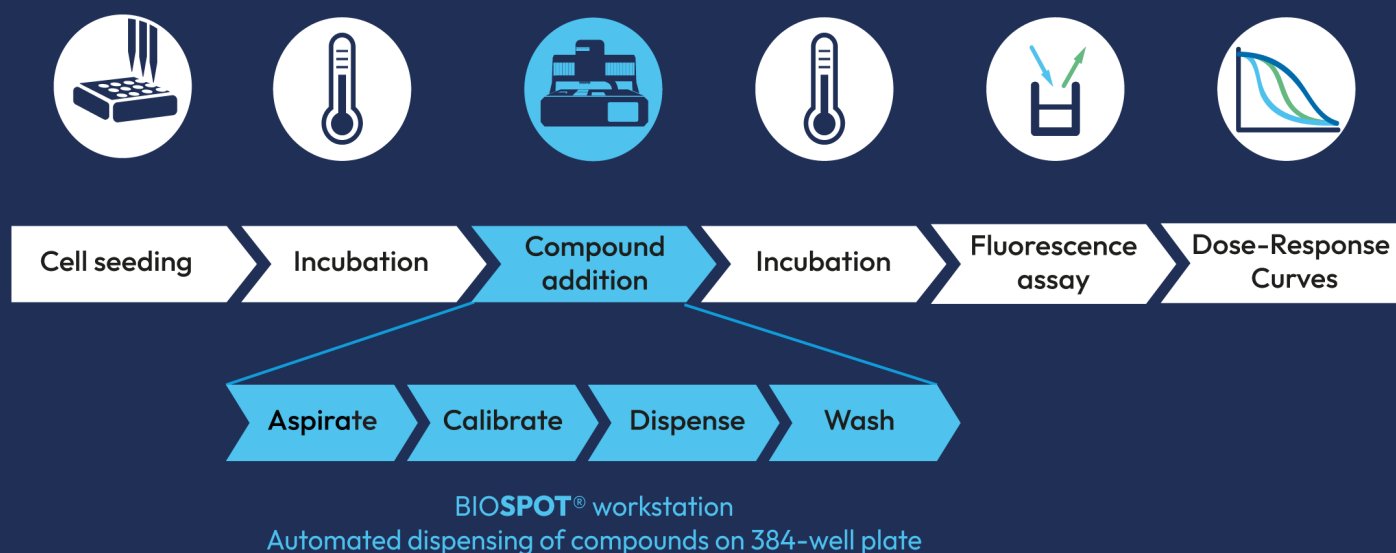


Figure 4: Visual Workflow.

The complete workflow from cell seeding to generation of the dose-response curves is illustrated. In light blue, the automated compound addition is highlighted, which encloses the automated aspiration, calibration, dispensing and washing steps.

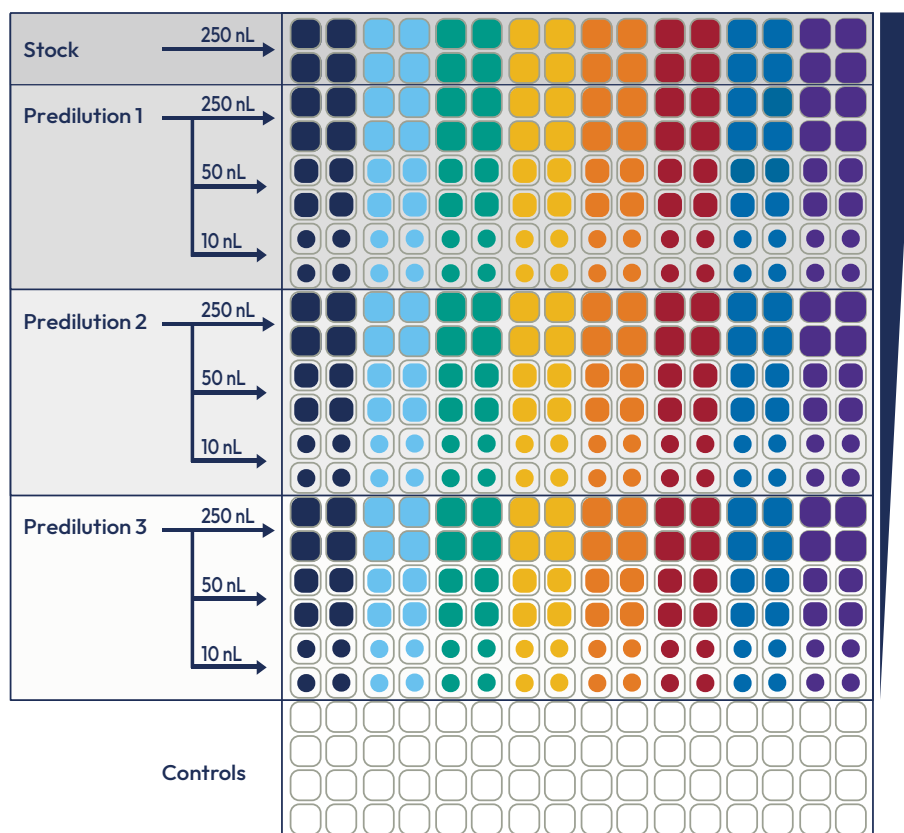


Figure 5: Pipetting Scheme.

The parallel generation of dilution rows for eight compounds (indicated by different colors) is shown. From a 10 mM stock solution, three pre-dilutions were prepared and dispensed into a 384-well plate. Each concentration was dispensed in quadruplicates. The dark blue triangle at the right illustrate the decreasing compound concentration from upper to lower wells.

pure DMSO, followed by three cycles with water, and concluding with three additional cycles with pure DMSO to eliminate any residual water. This automated cleaning process guarantees the integrity of each compound's dispensed volume, maintaining robust and reproducible assay conditions.

Readout

The number of viable cells was assessed via Promega's CellTiter-Blue® Assay. Hereby, the dye resazurin is reduced by metabolic active cells to resorufin, a highly fluorescent dye with excitation maxima at 579 nm and emission maxima at 584 nm. Non-viable cells do not reduce resazurin due to their loss of metabolic activity. Hence, acquisition of the fluorescence signal of resorufin enables assessing the number of viable cells. After a compound exposure time of 48 h at 37 °C, 5 % CO₂, the CellTiter-Blue® reagent was added according to the Kit supplier's protocol, followed by a 4 h incubation time at 37 °C, 5 % CO₂. The fluorescent signal of resorufin was measured in a plate reader (VANTAStar, BMG LABTECH) with the following filters: excitation filter: 540 (20) nm, emission filter: 590 (20) nm).

Data evaluation

Fluorescence raw data was blank corrected by subtracting the concentration-scaled fluorescence from the average of the 4 blank replicates (wells containing media and compound only, without cells).

The data were normalized to the maximum effect using wells containing cells and media only (without toxins) as positive control. Then, the average of the 4 technical replicates was calculated and plotted with the standard deviation. The data was fitted to the Hill equation^[10] to obtain the EC₅₀ values (Figure 6).

Fits and plots were generated with python 3.13.0 in Visual Studio Code 1.96.4.

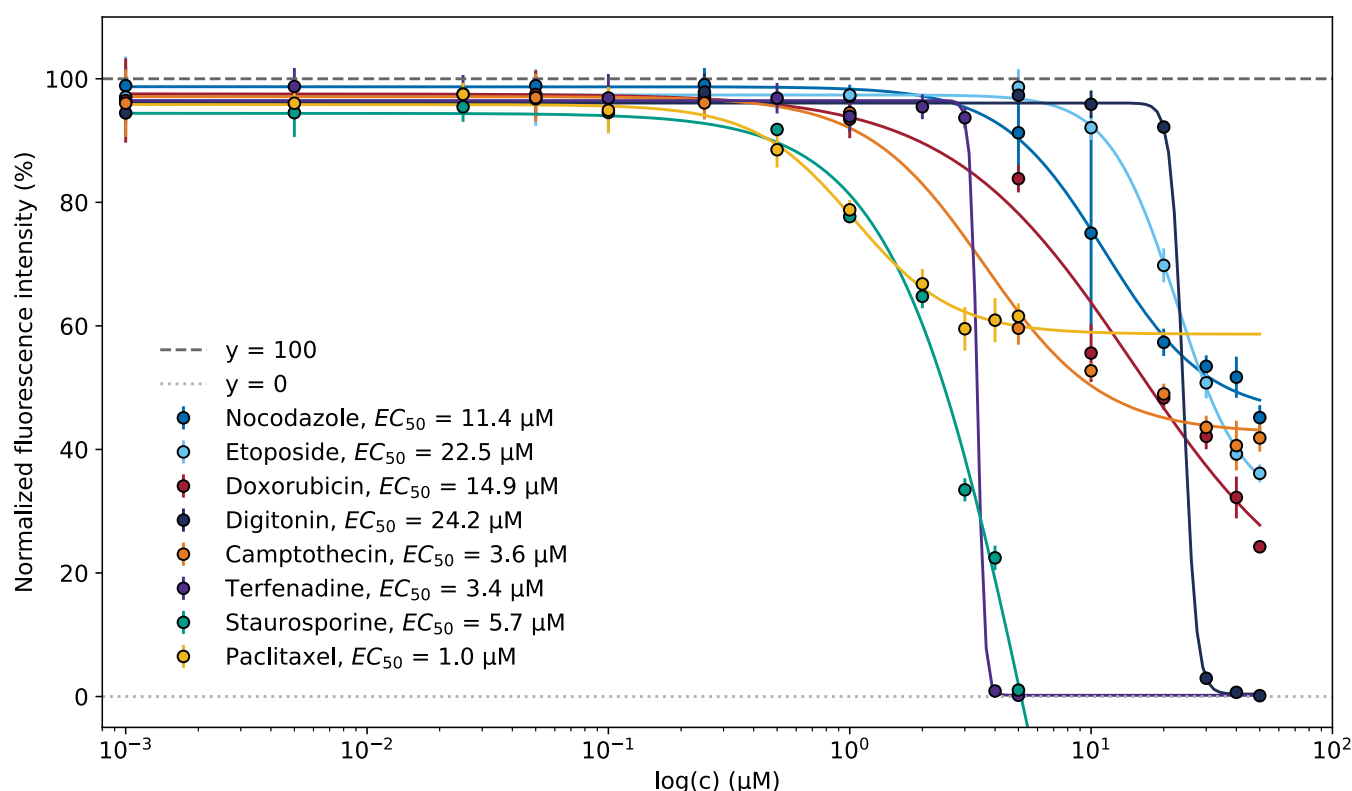


Figure 6: Resulting dose-response curves.

The eight dose-response curves resulting from the titration of the eight tested compounds onto HEK293 cells is shown. The viability of the cells was assessed via the CellTiterBlue assay (Promega). Blank-corrected data for the different compound concentrations were normalized to the mean fluorescence from cells without any compounds added (expressed in percent). The dots indicate the mean of 4 technical replicates with their standard deviation (error bars). Fits are depicted as solid lines. The resulting EC_{50} values for each compound are indicated in the figure legend. Note that the fit and EC_{50} value for Staurosporine are inaccurate, due to the missing plateau at its minimum.

Results

Figure 6 shows eight EC_{50} curves with their corresponding calculated EC_{50} values.

The precision and reproducibility of the dispensing process were critical in achieving consistent results.

All eight compounds exhibited the expected pharmacological behavior, with their relative potencies aligning with values reported in the literature. These findings validate the reliability of the BIOSPOT® nanoliter dispensing capabilities and its suitability to contribute in accurate compound characterization.

Conclusion

In conclusion, the high precision and accuracy of the BIOSPOT® workstation enables the automation of dose-response curve generation for up to eight compounds in parallel, while maintaining low DMSO concentrations — an essential factor for minimizing interference in cell-based assays. The system's flexibility extends beyond concentration range adjustments, as it allows for modification of drop size and dispensing layouts, offering versatility for different experimental needs. With the capability to handle up to six plates on deck, the system facilitates parallel dispensing into multiple target plates, which could include plates with different cell types, enhancing throughput and experimental complexity. Plates containing cells can be processed directly after removal from the incubator, without additional plate handling steps or restrictions based on plate volume or format. Automated aspiration and washing features and further optimized workflow, resulting in high walk-away time and reducing manual intervention. This blend of precision, flexibility, and automation makes the system an invaluable tool for reproducible drug screening.

References

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Requirements		
System requirements	Part Number	Provider
BIOSPOT® workstation	10186919 : 10023178	Hamilton
Labware requirements / Equipment	Part Number	Provider
Dispensing pipe 200-L, coated	10187065	Hamilton
Microtiter plates	781091	Greiner
VANTASTAR plate reader		BMG Labtech
Reagents	Part Number	Provider
CellTiter-Blue® Cell Viability Assay	G8080	Promega
Camptothecin	Cay11694	Cayman Chemical
Digitonin	TGM-T2721	TargetMol
Doxorubicin	Cay15007	Cayman Chemical
Etoposide	Cay12092	Cayman Chemical
Nocodazole	Cay13857	Cayman Chemical
Paclitaxel	Cay10461	Cayman Chemical
Staurosporine	Cay81590	Cayman Chemical
Terfenadine	Cay20305	Cayman Chemical
DMSO	A3672.0100	PanReac AppliChem

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