Application Note

HAMILTON

Next-Level Dose-Response Assays with BIOSPOT®: High Precision and Efficiency in Compound Concentration Curves

Authors: Julia Stifel [1], Thomas Clapes [1]

[1] BioFluidix, Georges-Köhler-Allee 302, 79110 Freiburg im Breisgau, Germany

BIO**SPOT**® simplifies dose-response curve generation with the PIPE**JET**® direct aspiration and dispensing capabilities at highest precision.

Introduction

Throughout lead identification and lead optimization phases of drug development, initial hit compounds are tested in dose-response assays (EC_{50}, IC_{50}, ADME Tox assays). Those assays are fundamental experiments in pharmacology and toxicology, aimed at understanding the relationship between the dose of a substance and the extent of the response it provokes in a biological system (i.e. efficacy and toxicity). Dose-response curves allow for the comparison between different substances and thus their clinical and therapeutic values.
Typically, compound stocks (usually 10 mM in DMSO) are cherry-picked from various storage formats, prediluted, and subsequently dispensed into assays plates. The concentration curves generally range from 7 to 11 data points, with each point increasing by half-logarithmic increments. The automated setup of concentration curves with the BIO**SPOT**® workstation allows for precise nanoliter dispensing without the use of multiple tips. Dispensing in nanoliter volumes opens up the possibility of achieving lowest possible compound concentrations while keeping the DMSO level to a minimum.

- Precise non-contact dispensing, adjustable from 7 - 15 nL per drop
- Trust the precision of every drop through automated volume calibration and QC
- Full flexibility on deck: up to 6 plates freely addressable with no need for plate handling or inverting
- Sustainable use of consumables with the optional pipe cleaning, which eliminates the need to manually replace pipes and allows them to be reused

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.**

Method

The automated dose-response assay was performed using a commercial fluorometric kit.

Figure 2. PIPE**JET**® modules, including pipes (yellow) inside the pipe guides (black), connected to the liquid reservoir tubing

System Description

BIOSPOT® workstation

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

PIPE**JET**®

Each PIPE**JET**® can independently aspirate liquid from any plate on-deck and precisely dispense nanoliter volumes.

SMART**DROP**®

The integrated SMARTDROP® technology employs high-speed cameras to capture the drop mid-flight, ensuring precise volume calibration across various liquid types. This allows for immediate adjustments and quality control, delivering droplets ranging from 2 to 70 nanoliters (pipe dependent) with a resolution of 0.1 nanoliters (see figure 3).

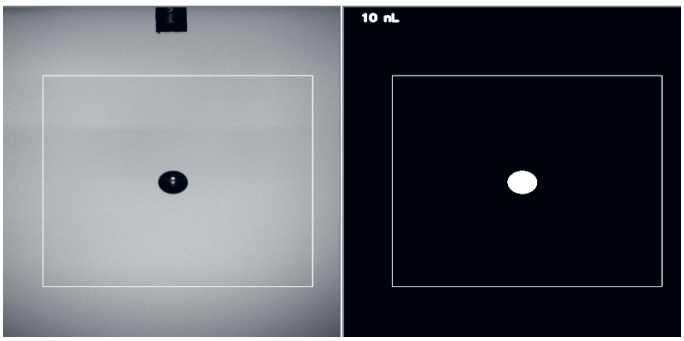

Figure 3. SMART**DROP**® technology capturing the drop mid-flight to ensure precise volume calibration across various liquid types.

Kit Description

Cyclooxygenase 2 (COX-2) or prostaglandin-endoperoxide synthase (PTGS) is an enzyme converting arachidonic acid to prostaglandin H2, which is involved in pain and inflammatory processes. A specific inhibition of COX enzymes is therapeutically relevant to treat these symptoms and optimize therapies to lower possible adverse effects.

The COX-2 Inhibitor Screening Assay Kit (82210) BPS Bioscience was used to measure dose-dependent inhibitor activity of the known COX-2 inhibitor Celecoxib (SML3031-10MG Sigma Aldrich). Celecoxib was manually diluted in DMSO to a concentration of 10 mM and used as a stock solution. Arachidonic Acid (Cayman Chemical #90010.1) was added to the assay as a substrate.

Workflow

Dispensing of 11-point half-log

The BIO**SPOT**® was used to create concentration curves of Celecoxib in black 96-well plates (provided with the kit), resulting in a 11-point half-log concentration curve. The pipetting scheme is shown in Figure 4. The 10 mM compound stock solution was aspirated, and the system was calibrated using the SMART**DROP**® feature to a final drop volume of 10 nL per drop. The stock solution was dispensed into DMSO prefilled wells (200 µL) on the intermediate plate to yield 2 predilutions with the concentrations of 100 µM (2 µL) and 1 µM (20 nL), respectively (see Figure 5). In addition to preparing predilutions, the concentrated stock solution was dispensed into the respective wells of the assay plate to result in the three highest doses of the final concentration curve. Both predilutions were aspirated from the intermediate plate, calibrated to a drop volume of 10 nL, and dispensed into the respective wells on the assay

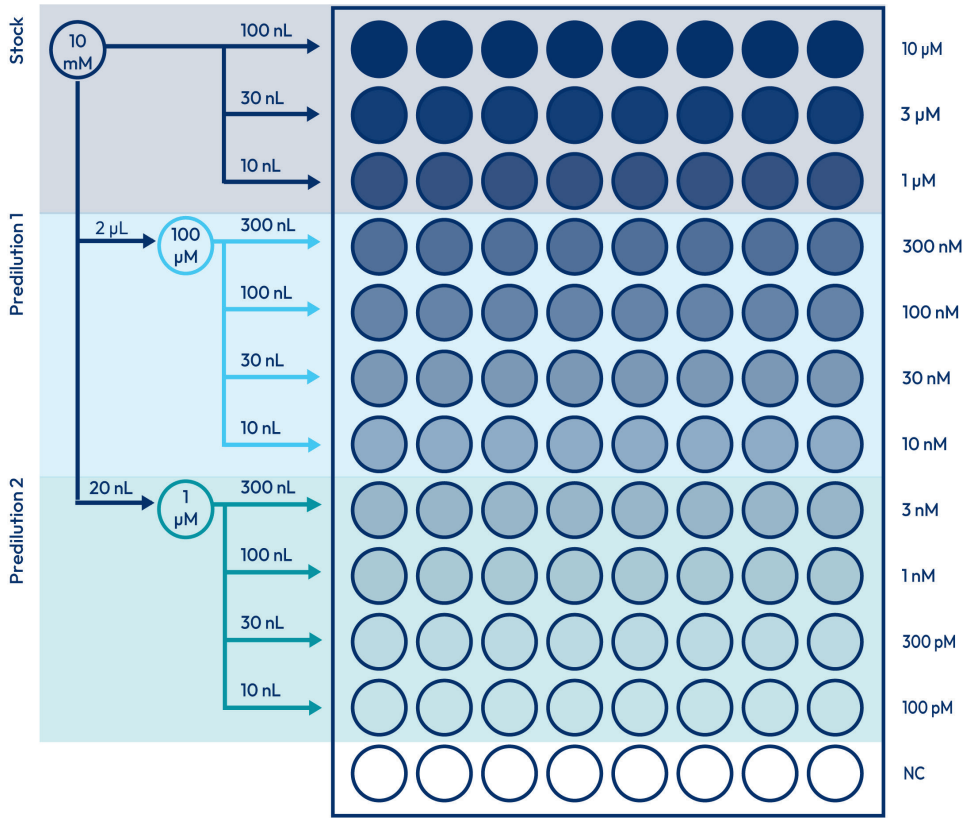

Figure 4. Pipetting scheme from a 10 mM Stock solution to a 11-point half-log concentration curve, including two predilution steps. NC, negative controls.

plate. In a last step, DMSO was aspirated from the intermediate plate to backfill (add DMSO to a total volume off 300 nL per well) all dispensed wells. A constant DMSO level in all wells is essential to exclude the effect of varying DMSO concentrations interfering with the effect of the compound.

Targeted concentration curves

To achieve more detailed data in the region of the curve where the reaction is most responsive (around the expected IC_{50} value, which indicates the concentration needed to inhibit a response by half), the concentrations were set in smaller (one-third of a logarithm) increments. From the 10 mM Stock, one predilution (100 µM) was prepared as described above. Both Stock solution and the predilution were aspirated, calibrated to a drop size of 10 nL, and dispensed into the respective wells. All wells were backfilled with DMSO to ensure constant levels in each well.

Visual Workflow

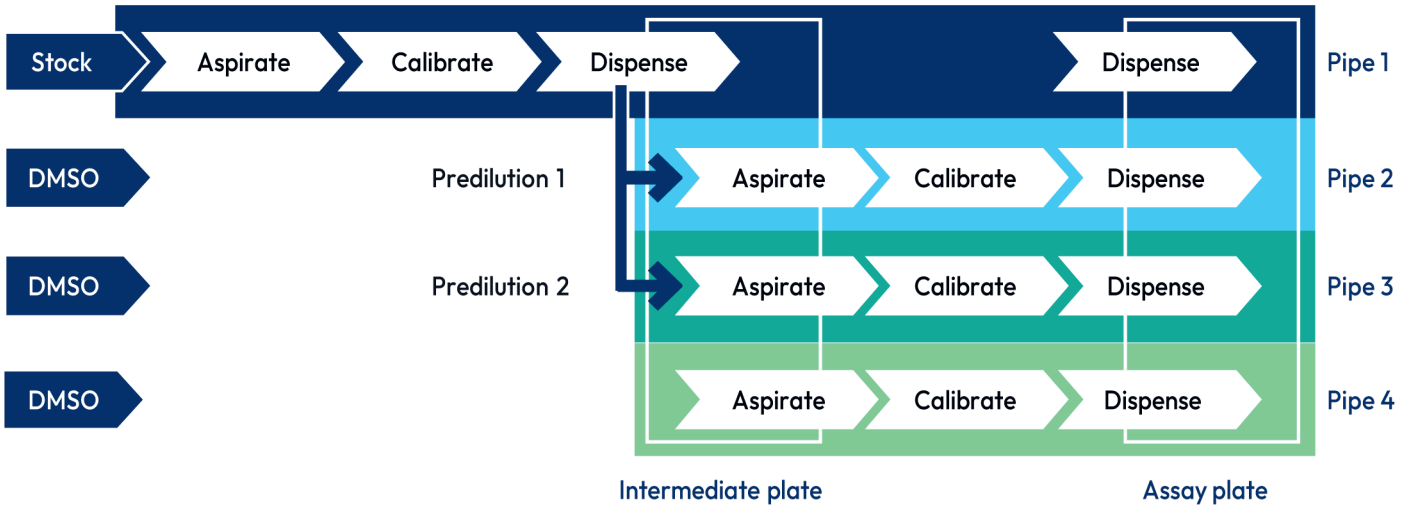

Figure 5. Visual Workflow. Illustrates the complete workflow for creating a concentration curve from a stock solution, including a DMSO backfill.

Pipe washing

To evaluate the effectiveness of washing and reusing a dispensing pipe, a targeted concentration curve was generated using a single pipe, providing a comparison to the previously described experiment. Figure 6 illustrates the workflow of this experiment. The optimized washing procedure for DMSO involved three cycles with pure DMSO, followed by three cycles with water, and concluding with three additional cycles with pure DMSO to eliminate any residual water. The results of this comparative experiment are presented in Figure 8.
Following the compound dispensing performed by the BIO**SPOT**®, all kit reagents were added manually, according to the Kit supplier's protocol.
Fluorescent signals were measured in a plate reader (BMG Labtech Clario Star, Ex 535 nm Em 590 nm).
All experiments were performed with 200-L-C Pipes, allowing drop sizes in a volume range between 7 nL and 15 nL to be dispensed. As aspiration tubes, PTFE tubing was installed to ensure material compatibility with DMSO.

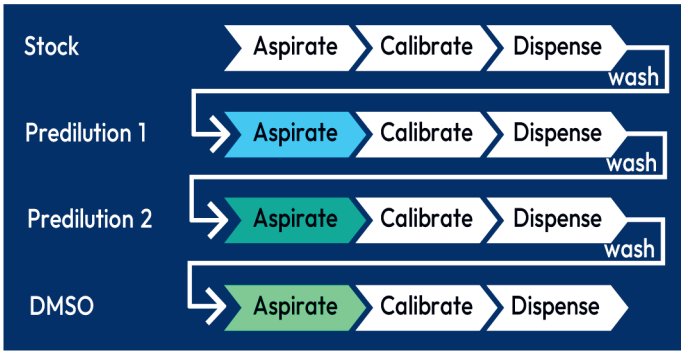

Figure 6. Dispensing workflow including pipe washing. All steps in this workflow were dispensed in the same pipe.

Results

The full 11-point half-log dose-response curve is shown in Figure 7. Fluorescence levels were normalized to relative COX-2 activities, based on signals from both negative and positive controls. To achieve higher data resolution on the curve's slope-area, a second experiment was conducted with smaller concentration steps (10 to 1000 nM), resulting in an IC50 value of 113 nM.

Both methods—using dedicated pipes (white) and reusing a single pipe after washing (green)—produced the same inhibition profile, with no observed carryover affecting the concentration curve.

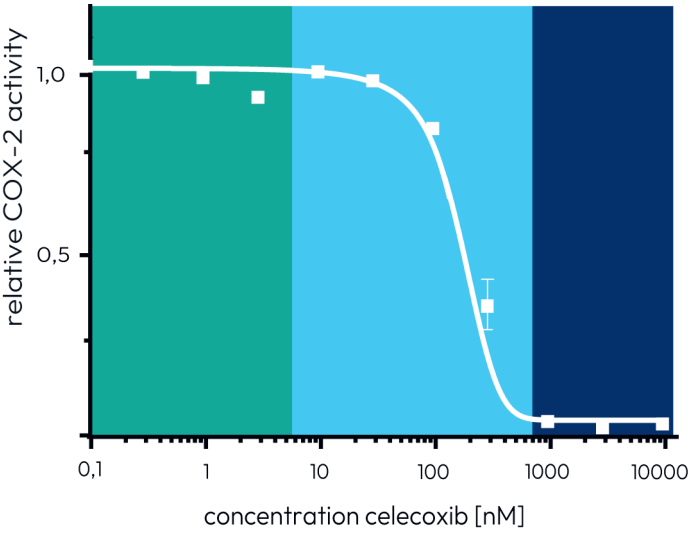

Figure 7. Relative COX-2 activity for Celecoxib concentrations ranging from 0.1 nM to 10 mM in half-logarithmic steps. Data are shown as the average of duplicates. The background colors illustrate the respective predilution used to yield those concentrations.

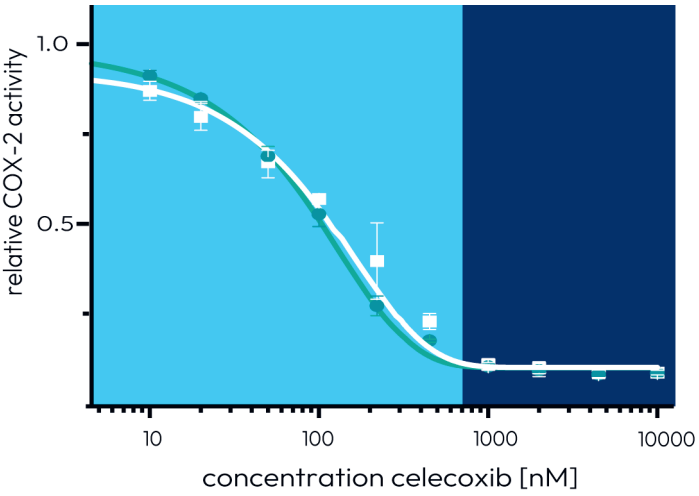

Figure 8. Relative COX-2 activity for targeted Celecoxib concentrations, comparing the workflows including pipe washing (green) and using a fresh pipe for each aspiration step (white). Data are shown as the average of duplicates. The background colors illustrate the respective predilution used to yield those concentrations.

Conclusion

The BIO**SPOT**® Workstation delivers precise and reproducible 11-point half-logarithmic dose-response curves.

The capacity on the deck of the BIO**SPOT**® can accommodate up to six freely addressable microtiter plates without the need for plate handling or transfer. This permits several compound plates to be prepared for downstream processing in different assays in a single run.

With the aspiration feature, both the preparation of predilutions as well as the final dispensing into the assay plate can be handled by the BIO**SPOT**®, presenting an automated solution for the preparation of assay-ready compound plates.

With a washing procedure tailored to the assay requirements, the BIO**SPOT**® demonstrated efficient processing of all necessary dispensing steps for the generation of concentration curves using a single substance. Given the availability of eight channels, the system has the potential to handle up to eight substances in parallel. This approach significantly increases walk-away time by automating the cleaning process and eliminating the need for manual exchange of pipes between reagents. While the washing procedure must be optimized for a broader range of reagents to expand the system's versatility and applicability, automating these processes and aspirating compounds directly into microtiter plates enhances productivity and streamlines workflows in various laboratory settings.

Requirements		
System requirements	**Part Number**	**Provider**
BIO**SPOT**® - base configuration	BSC-30101	Hamilton
Confocal illumination for TOP**VIEW** system	BSC-30103	Hamilton
SMART**DROP**® BS	BSC-30102	Hamilton
Ionizer – printhead integrated	BSC-30104	Hamilton
PIPE**JET**® nanoDispenser - low volume	PJ-90011	Hamilton
PIPE**JET**® nanoDispenser – Standard	PJ-90012	Hamilton
Aspiration option	BSC-30110	Hamilton
Pipe wash option	BSC-30111	Hamilton
Labware requirements	**Part Number**	**Provider**
Dispensing pipe 200-L, coated	PJ-20051	Hamilton
Reagents	**Part Number**	**Provider**
COX-2 Inhibitor Screening Assay Kit	82210	BPS Biosciences
Celecoxib	SML3031-10MG	Sigma Aldrich
Arachidonic Acid	90010.1	Cayman Chemical
DMSO	5.89569	Sigma Aldrich

HAMILTON

To find a representative in your area, please visit:

hamiltoncompany.com/contact
infoservice@hamiltonrobotics.com

United States
+1-775-858-3000
United Kingdom, Ireland
+44 121 272 92 80
Brazil
+55 11 95914 5000
China
+86 21 6164 6567

Denmark, Norway, Sweden, Finland
+46 8410 27 373
France
+33 184 008 420
Germany, Austria, Switzerland
+49 89 248 804 804

Benelux
+31 40 209 178 0
Italy
+39 039 930 06 06
Japan
+81 3 6435 6850
Spain, Portugal
+34 930 186 262