Application Note

Bravo & Seahorse XF

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Maximizing Efficiency and Performance of Seahorse XF Assays with the Bravo Automated Liquid Handling Platform

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Abstract

It is well accepted that manual sample preparation steps can influence assay data quality, especially with cell-based assays. Manual sample preparation (including pipetting) can introduce errors and can require significant amounts of time. To avoid potential error and reduce hands-on preparation time, the use of automated liquid handling for Agilent Seahorse XF assay sample preparation is presented. When used in a routine assay workflow an Agilent Bravo Automated Liquid Handling Platform (Bravo): 1) reduced variability due to manual errors, 2) improved consistency and reproducibility within and across microplates, 3) reduced hands-on time for XF assay preparation. Overall, automating the liquid handling steps of a Seahorse XF assay resulted in improved efficiency of the sample preparation workflow and consistency of resulting data, especially in the context of drug discovery applications such as routine compound assessment and dose-response studies.

Introduction

Cell-based assays often require several liquid handling steps that can influence data quality. Lack of consistent sample preparation can result in unacceptable variability across wells, plates, or replicate assays. These pipetting steps can also be labor-intensive, especially when performing multiplexed and/or large numbers of replicate assays, as is often required in drug discovery projects.

For example, although a skilled manual operator can perform cell washing consistently, there is still an increased chance that manually washing cells will result in damage to the monolayer. This damage can result from common errors, such as those illustrated in Figure 1. These include damage or loss of cells by scratching of the monolayer surface (panel B), dislodging weakly adherent cells by forceful pipetting (panel C), or removal of too much media that exposes the monolayer to air and results in damage or detachment (panel D). Manual pipetting also has potential to introduce bubbles that may damage cells or impact the final well volume.

To address these issues when performing Seahorse XF assays, several key manual pipetting steps can be automated with the Bravo Automated Liquid Handling Platform. Performing these liquid handling steps via the Bravo provides value for the researcher with respect to data quality and reduction in assay preparation (hands-on) time. Both of these advantages allow for increased productivity due to more consistent data as well as increased "walk away" time during the XF assay.

In this Application Note, methods are presented for automating three key liquid handling steps associated with XFe96 assay preparation: washing cells, preparing injection solutions, and loading these solutions into the XFe96 sensor cartridge. Examples are then provided illustrating each automation step, including assay data, as well as estimated gains in “walk away” time.

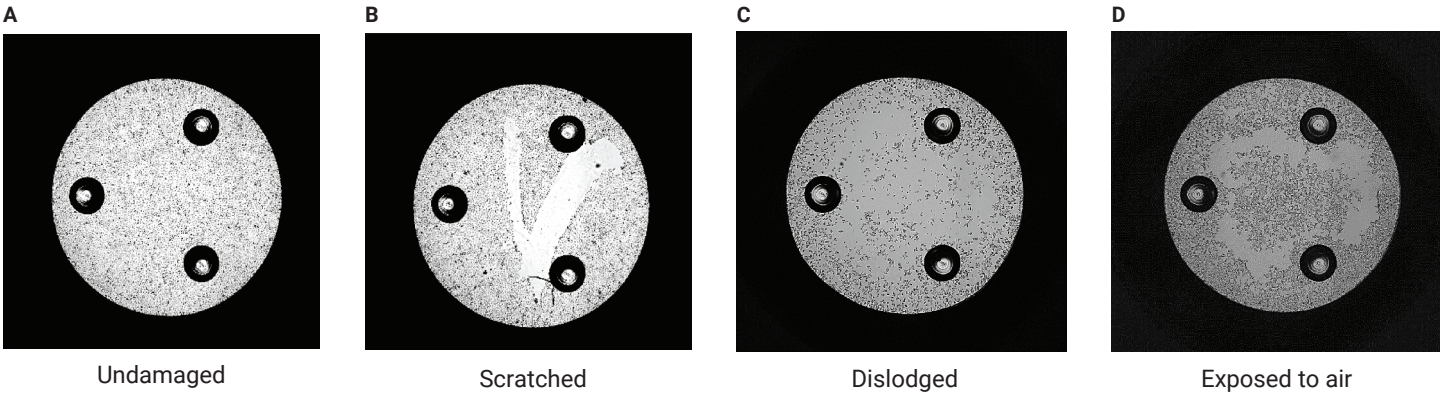


Figure 1. Brightfield images of single wells in an Agilent XF96 microplate depicting damage to the cell monolayer. A) Undamaged cells. B) Scratched with a pipet tip. C) Dislodged during aspirate or dispense. D) Detached due to exposure to air.

Materials and methods

Cell culture

HEK293 or HepG2 cells were seeded in XF96 cell culture microplates at a density of 3.0×10^4 or 1.5 to 2.0×10^4 cells per well, respectively, and were cultured overnight at 37 °C 5% CO_2. HEK293 cells were grown in DMEM (Gibco 11885084) supplemented with 10% FBS and 2 mM GlutaMAX (Gibco 35050061) and HepG2 cells in EMEM (ATTC 30-2003) supplemented with 10% FBS. After 24 hours, the cells were prepared for the XF assay.

Normalization

Hoescht nuclear dye (Thermo Scientific, PI62249) was included with the final injection in all assays at a concentration of 20 µg/mL (2 µg/mL in the well) and microplates were imaged with a BioTek Cytation 1. Nuclei were counted in each well and the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) data were normalized to these nuclear counts. For an in-depth description of the Seahorse XF Imaging and Normalization system, please refer to Normalization of Agilent Seahorse XF Data by In-situ Cell Counting Using a BioTek Cytation 5.

Bravo setup and performance testing

All assays were performed using a Bravo configured for use with XFe96 Analyzer associated consumables. This configuration has heated blocks in positions four and six on the Bravo deck. Bravo protocols for each workflow element, including preset aspiration and dispensing speeds and heights (see Appendix Table A1 for details) were implemented via Bravo Seahorse Assay Workbench software. Agilent labware (reservoirs and reagent plates) for the Bravo and XFe96 consumables were used as specified in Table 1. All XF assays were performed using XF assay media composed of Agilent Seahorse XF DMEM pH 7.4 media + 10 mM glucose, 1 mM pyruvate, and 2 mM glutamine. Any manual pipetting steps were performed in accordance with recommended XF assay methods and practices.

Table 1. Agilent labware and consumables required for each automation step.

Agilent Labware and Consumables	Part Number	Quantity Required per XFe96 Assay		
		Cell Washing	Compound Dilution	Cartridge Loading
Agilent 24-Column Reservoir (Polypropylene, 3.25 mL/Column, Pyramid Base Geometries)	201296-100	–	–	1
Agilent 96-Well Storage/Reaction Microplate (Ultrahigh Purity Polypropylene, 1 mL/Square Well, Conical Bottoms)	201276-100	–	2	–
Agilent Reservoir (Polypropylene, 86 mL, 96 Pyramids Base Geometry)	201254-100	2	1	–
Agilent Tip Box, 250 µL, 96 in Rack, Compatible with Bravo 96LT Head (Full)	19477-002	1	2	1
Agilent Tip Box, 250 µL, 96 in Rack, Compatible with Bravo 96LT Head (Empty)		–	1	1
Agilent Seahorse XFe96 FluxPak*	102416-100	1 cell plate (96-well Seahorse cell plate)	1 XFe96 cartridge with utility plate (Seahorse XFe96 cartridge with utility plate, 384 ports)	
Seahorse XF DMEM pH 7.4 Media	103575-100	90 mL	40 mL	20 mL
Seahorse XF Glucose (1.0 M Solution)	103577-100	0.9 mL	0.4 mL	0.2 mL
Seahorse XF Pyruvate (100 mM Solution)	103578-100	0.9 mL	0.4 mL	0.2 mL
Seahorse XF L-Glutamine (200 mM Solution)	103579-100	0.9 mL	0.4 mL	0.2 mL
XF Cell Mito Stress Test (Source of Oligomycin, FCCP, and Rotenone/Antimycin-A)	103015-100	–	1	1
Glycolytic Rate Assay Kit (Source of 2-DG)	103344-100	–	1	1

* The Agilent Seahorse XFe96 FluxPlak is discontinued and replaced by XFe96/XF Pro FluxPak, part number 103792-100. The method described in this application note is also applicable for use with the XF Pro M FluxPak, part number 103775-100.

Cell washing

HEK93 cells were used as a model of a loosely adherent cell line to demonstrate the ability of the Bravo to wash cells without damage to the monolayer or loss of cells (described in Figure 1). The Bravo deck configuration and labware used for cell washing is shown in Figure 2. Briefly, cells were washed by aspirating the growth medium and replacing it with assay medium two times, leaving a final well volume of 180 µL. Three basal measurements were made in a Seahorse XFe96 Analyzer and evaluated.

Injection port loading

Stock solutions of XF Cell Mito Stress Test reagents oligomycin, FCCP, and antimycin-A/rotenone were manually prepared and dispensed into an Agilent 24-column reservoir (part number 201296-100). The Bravo was then used to transfer 20, 22, or 25 µL of these solutions into the A, B, and C ports, respectively, of an XFe96 sensor cartridge. The Bravo deck configuration for XFe96 sensor cartridge loading is shown in Figure 3. All cell washing steps for this series of assays were performed by the Bravo as described previously.

Injection solution preparation – FCCP custom dilution

Dilutions were performed in Agilent 96-well polypropylene storage plates (part number 201276-100). The Bravo deck configuration for compound dilution and XFe96 sensor cartridge loading is shown in Figure 4. A custom 10-step dilution series of FCCP, 2 to 20 µM, in increments of 2 µM was performed. A common stock of 100 µM FCCP stock solution was manually prepared in XF assay media in the library source plate. Dilutions of FCCP were performed by Bravo into the dilution plate directly from the 100 µM stock solution, followed by automated loading into ports "B" of an XFe96 cartridge. All FCCP treatments were performed in the presence of 1 µM oligomycin. The Bravo was used to load 20 µL of a 10 µM oligomycin injection solution into all A ports and 25 µL of a rotenone/antimycin A solution (5.0 µM each) into all C ports to complete the standard XF Mito Stress Test (MST). This FCCP dose-response assay was repeated on a separate day (two replicates).

Injection solution preparation – Antimycin A serial dilution

To evaluate the serial dilution protocol on Bravo, a 100 µM antimycin A (AA, Sigma A8674-25) stock solution was prepared from 50 mM AA solution in DMSO diluted with XF assay media. Subsequent dilutions were performed by the Bravo platform in an Agilent 96-well storage plate (part number 201276-100). Eight independent, identical dilution series of AA were prepared in parallel on the same microplate. Each dilution series consisted of a 10-step

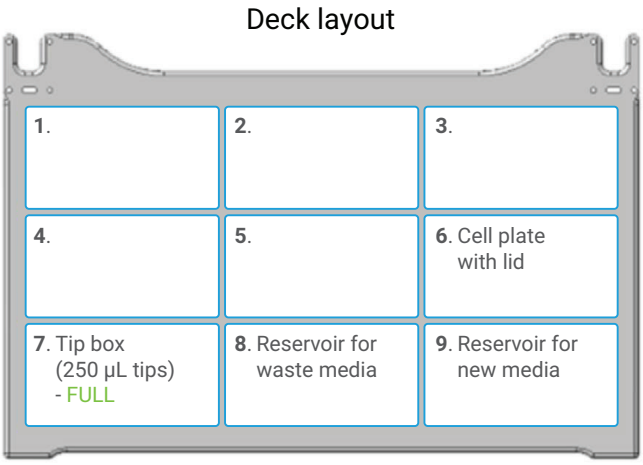


Figure 2. Agilent Bravo deck configuration and labware used for cell washing.

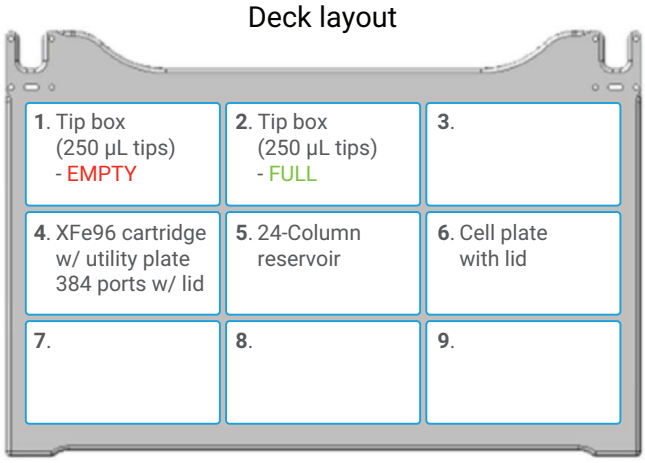


Figure 3. Agilent Bravo deck configuration and labware used for injection port loading.

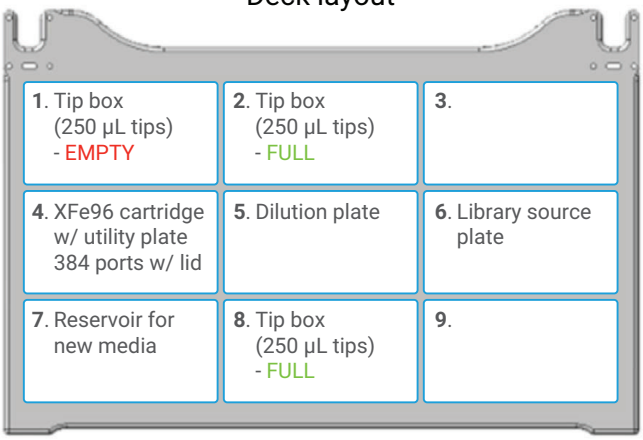


Figure 4. Agilent Bravo deck configuration and labware used for the dilution series.

two-fold serial dilution, left to right, resulting in injection port concentrations ranging from 10 to 0.02 µM. AA dilutions were loaded into ports "A" of the XFe96 cartridge using the Bravo. Three independent assays were performed.

Compound Library Screen for mitochondrial and glycolytic inhibitors

A total of 72 compounds from the Selleck-Pfizer Library (Selleckchem.com, part number L2400, Pfizer Licensed compound library) were screened for mitochondrial and glycolytic inhibi-tion. For this small screen, the Bravo was used to 1) wash cells, 2) perform 100× dilutions of the compound library from a 96 well source plate, and 3) dispense 20 µL of the diluted compound solutions into the XFe96 sensor cartridge ports A. The Seahorse XFe96 assay consisted of three rate measure-ments (basal OCR and ECAR), followed by injection of the test compounds (10 µM final) and nine more rate measurements. OCR and ECAR values from the final measurement (number 12) were used for determining inhibitory activity.

Three XF assays were performed in parallel, generating technical replicate microplates. This method was repeated three times, generating a total of nine technical replicates (i.e., nine individual microplates). Z' for data quality was generated using 0.5 µM antimycin A/rotenone cocktail (AA/Rtn) as the OCR inhibition positive control, 50 mM 2-deoxyglucose (2-DG) for the ECAR inhibition positive control, and 0.1% DMSO (final) as the vehicle control.

Results and discussion

Hoescht nuclear dye (Thermo Scientific, PI62249) Seahorse XF assays have common sample preparation elements regardless of the specific application or kit being used:

a. Seeding cells in the XF96 cell culture microplate
b. Washing cells (exchanging growth media for assay media)
c. Compound preparation (dilutions)
d. Compound port loading into the XFe96 sensor cartridge

These workflow steps are routine, involve substantial pipetting, and have the potential to add error to assays; as such, they are good candidates for automation.

This project focused on steps b to d to assess the utility of Bravo automation to perform post culture live cell sample preparation for Seahorse XFe96 assays. Automation of cell seeding with Bravo has previously been shown to improve assay robustness (Gestin *et al.*). Although the Bravo can fit in a tissue culture hood, such an arrangement was not implemented in this laboratory, so the performance of automated cell seeding was not assessed.

A high-level workflow for a Seahorse XF assay is depicted in Figure 5.

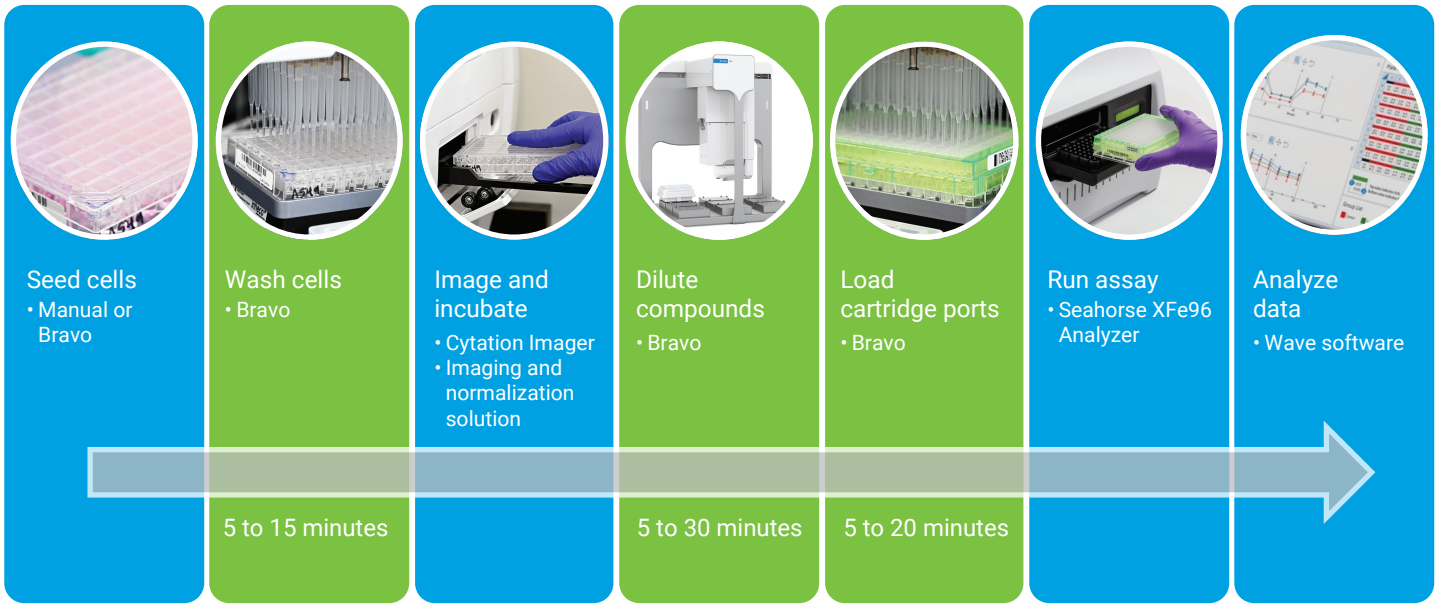


Figure 5. General Agilent Seahorse XF Assay workflow. Sample preparation steps automated with Bravo are shown in green: washing cells, preparation of injection solutions, and loading of Seahorse XFe96 sensor cartridges. Total hands-on time savings of 20 to 60 minutes per plate.

Washing cells

In XF assays using adherent cells, cells are washed before the XF assay to remove growth media constituents and provide the cells XF assay media. To validate that cells could be washed by an automated system consistently and without damage to or loss of cells, weakly adherent HEK293 cells were tested. When washing cells, it is possible to dislodge weakly adherent cells by dispensing media too quickly and/or accidentally touching cell monolayers with pipette tips. The Bravo was programmed for a specific height above the well bottom to avoid touching the cell layer. In addition, the aspiration and dispensing of assay media was programmed at rates that minimize the risk of dislodging cells from XF96 cell culture microplates.

To evaluate automated cell washing, an XF assay measuring basal respiration (Oxygen Consumption Rate or OCR) and acid (H^+) production (Extracellular Acidification Rate or ECAR) was performed using HEK293 cells (Figure 6). The data presented illustrate that the Bravo performed the cell washing step consistently. In the example shown, normalized rates using the Bravo for washing were not significantly different per well (Figure 6B). Further, when the assay was repeated over three days, the data show consistent ECAR values with low standard error for each day. This data indicated that the integrity of the cell monolayer was well-maintained when the Bravo was used to wash cells (Figure 6C). In addition, the Bravo successfully washed suspension cells that were adhered to XF96 cell culture microplates with Cel-Tak immediately before the cell washing process (data not shown).

The Bravo minimizes the likelihood of damage to the cell monolayer (as illustrated in Figure 1) by controlling both the height of the tips in the well and the speed of liquid aspiration or dispense. This results in improved consistency in residual volume in the well, prevents scraping off or dislodging cells from the well surface, and prevents the formation of air bubbles. In addition, the media and cell plate are kept at 37 °C on the Bravo deck throughout the cell wash. This minimizes temperature variations of the cells and assay medium thus further decreasing the risk of added variation in the assay results.

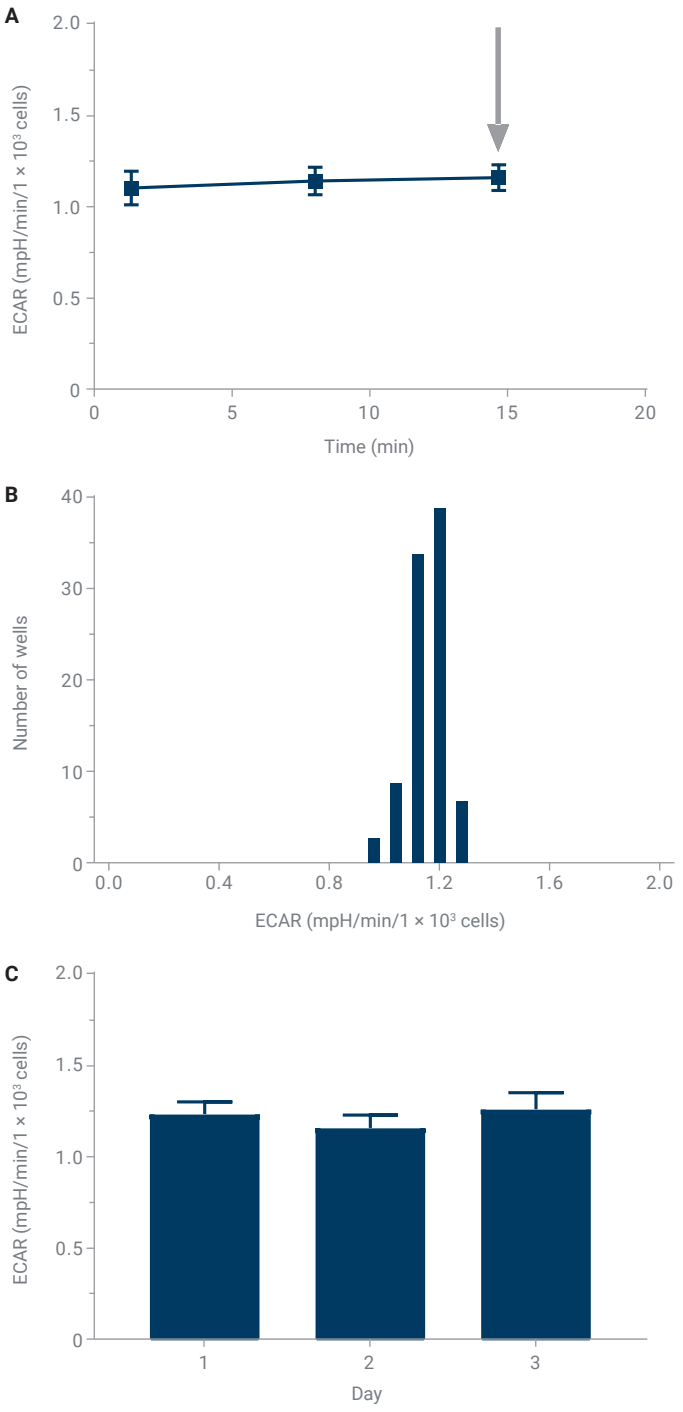


Figure 6. Automated cell washing protocol test. Microplates were seeded with HEK293 cells and automated cell washing was performed using an Agilent Bravo Automated Liquid Handling Platform, followed by an Agilent Seahorse XF assay. The third rate measurement was used for data comparison. (A) Basal ECAR (no injections) XF assay with normalization. The arrow indicates the data used in panels B and C. (B) Histogram of ECAR values (measurement 3) for a single XF96 microplate (n = 92 wells). (C) ECAR values (measurement 3) for three replicate assays performed over three days. Data are presented as the mean ±STDEV (n = 92).

Injection port loading

Seahorse XF assays generally require one or more injection ports of the sensor cartridge to be loaded before the assay. The volume of injection solution loaded should be identical in a given series of injection ports (e.g. all "A" injection ports, etc.) to ensure accurate and precise injection of the solution, and hence provide the same resulting drug concentration in each well. In addition, care must be taken to load the ports gently so as not to force liquid through the bottom of the injection port.

To validate that injection solutions are loaded accurately and consistently with a Bravo, an XF Cell Mito Stress Test (MST), consisting of three sequential injections, was performed. Maximal respiration was analyzed (Figure 7A, arrow) as this parameter is sensitive to small variations in FCCP concentration.

Figure 7 illustrates that the Bravo performed accurately and consistently when loading injection solutions into the sensor cartridge ports. The maximal response, a sensitive metric for small variations in the concentration of FCCP, showed near identical respiration rates and responses to MST injections (Figure 7B) on a per well basis. Finally, when the assay was repeated another day, the data showed consistent OCR values with low standard errors for each day (Figure 7C). Taken together, this illustrates that the Bravo consistently transferred the injection solution to the desired port in a manner supporting complete injection of the solution from the port.

Accurate and consistent port loading is essential to high-quality data. While a skilled manual operator can load ports accurately and consistently, there is still the potential for error and hands-on skills vary considerably from person to person. Incorrect loading of the ports can result in poor injection (injection failure) or leaking of compounds from the port before they are injected. A partial injection or incorrect volume will result in a lower than desired concentration in the well. A leak will result in an unexpected response earlier in the assay than intended, usually rendering those data points unsuitable for analysis. The Bravo consistently performs this step for any XF assay by dispensing the accurate volume without creating air bubbles or use of excessive pressure. It also further reduces the risk of user errors such as cross-contamination of pipette tips and/or ports.

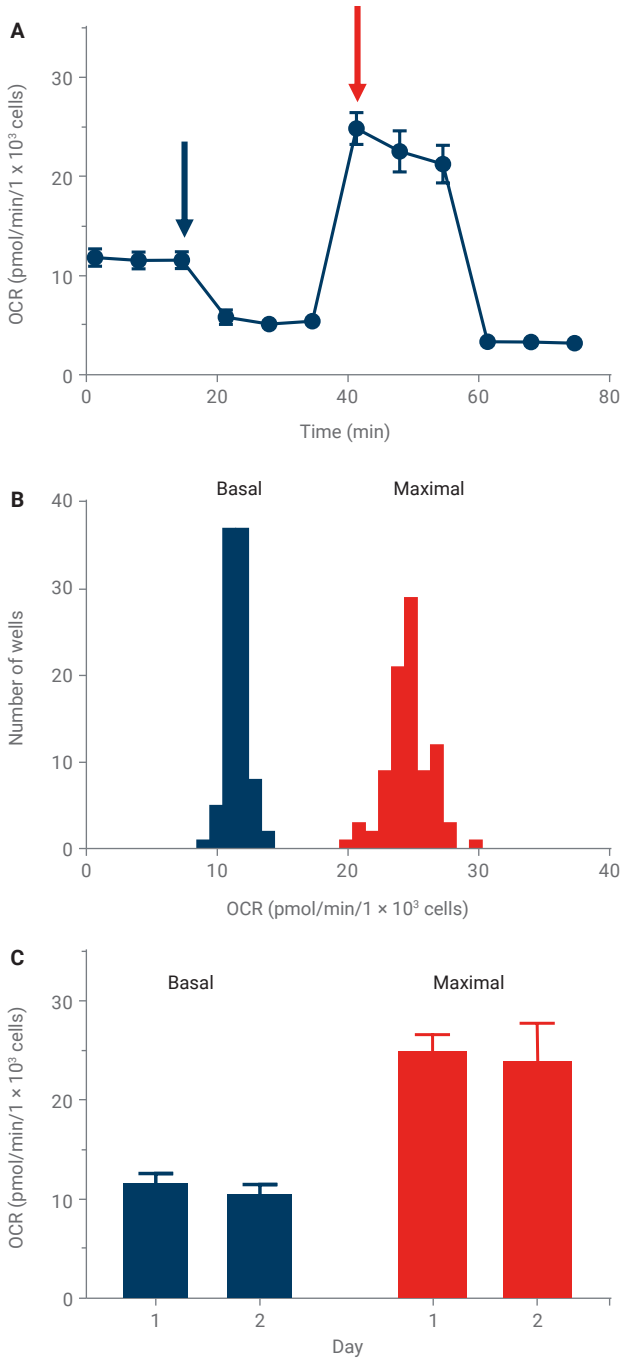


Figure 7. Automated injection port loading evaluation. HepG2 cells were seeded in Agilent Seahorse XF96 cell culture microplates and assayed after 24 hours of growth. MST injection solutions of oligomycin, FCCP, and rotenone/antimycin A were prepared and loaded into the XF cartridge. (A) Cell Mito Stress Test results, arrows designate basal (blue) and maximal (red) respiration data used panels B and C. (B) Histogram of normalized basal and maximal OCR values (measurements 3 and 7) for a single XF96 microplate (n = 92 wells. (C) Mean normalized basal and maximal OCR values (measurements 3 and 7) for two independent assays. Data is presented as the mean ±STDEV (n = 92).

Serial dilutions and dose-response assays

When characterizing a series of compounds or drug candidates, typically a dose-response assay is performed to assess potency (e.g., IC_{50} or EC_{50}). The Bravo can facilitate this process by minimizing the hands-on requirements (and associated risks) of preparing and executing a complex dilution matrix assay.

Two assays designs were used to test the ability of the Bravo to generate dose-response matrices consisting of 1) technical replicates within a single microplate (Figures 8 and 9) and 2) technical replicates across several microplates (Figures 10 and 11).

As noted previously, cellular respiration rates are sensitive to small variations in FCCP concentration, especially at submaximal concentrations. Thus, a dose-response assay within a narrow range of FCCP concentrations is a robust test of the precision of the Bravo for the preparation of dose-response assays.

To test technical replicates within a single plate (Figure 8), HepG2 respiration was measured in response to 0.0 to 2.0 µM FCCP in 0.2 µM increments via an MST assay. Maximal respiration (Figure 9A, arrow) increases linearly within this narrow range, reaching maximal OCR at 1 µM FCCP, then declining slightly at 2 µM FCCP (Figure 9B). Each concentration elicits a distinct response with low CVs, demonstrating the precision of the Bravo when preparing this custom dilution series. When performed over multiple days (N = 2) the resulting data shows consistent responses to FCCP and resulting CVs from all doses are <10% (Figures 9B and 9C).

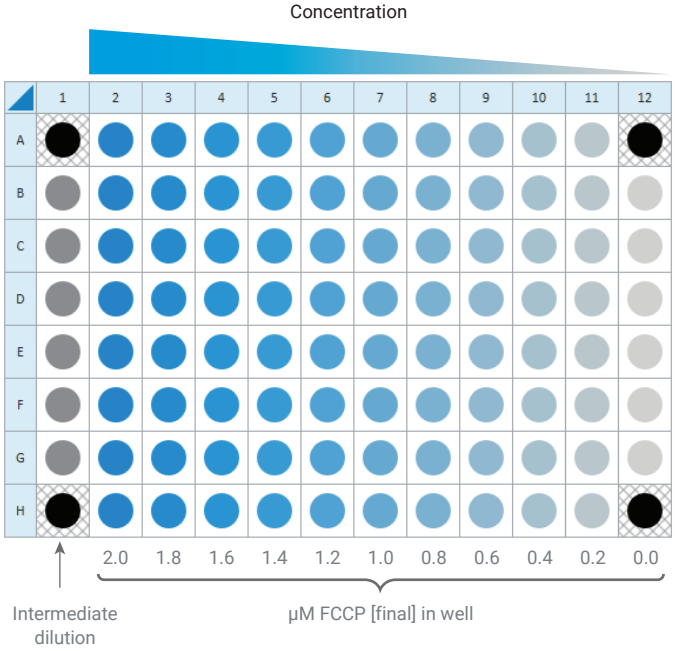
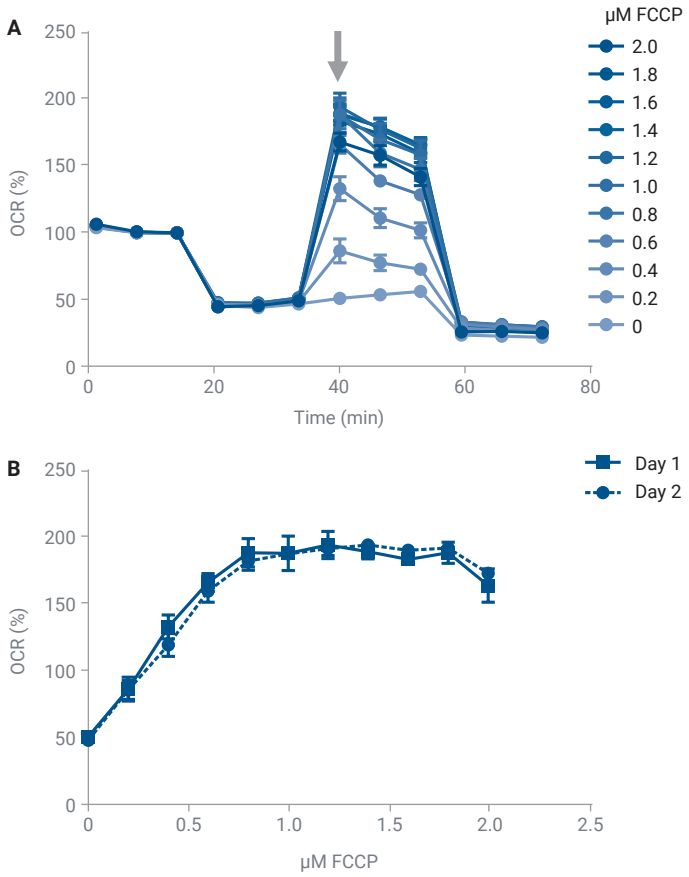


Figure 8. Plate layout for testing technical replicates within a single microplate. XF dose response assay design using a single compound (FCCP). The assay consists of a 10-step dilution for final FCCP concentrations of 0.0 to 2.0 µM, with eight technical replicates per concentration.



C

Day 1			µM FCCP	Day 2		
%OCR	STDEV	%CV		%OCR	STDEV	%CV
51.0	2.1	4.2	0.0	48.2	1.8	3.8
86.7	9.1	10.5	0.2	86.0	7.1	8.2
133.3	9.1	6.8	0.4	119.7	8.6	7.2
166.5	6.7	4.0	0.6	160.0	8.4	5.3
188.9	10.6	5.6	0.8	182.5	7.0	3.9
188.4	13.0	6.9	1.0	187.9	3.8	2.0
194.7	10.3	5.3	1.2	192.0	5.3	2.7
189.8	5.4	2.9	1.4	194.7	1.9	1.0
183.9	4.6	2.5	1.6	190.6	3.2	1.7
188.9	7.9	4.2	1.8	192.5	4.2	2.2
164.3	12.6	7.6	2.0	173.5	5.0	2.9

Figure 9. Technical replicates within a microplate. An FCCP titration was performed on HepG2 cells in a narrow range of concentrations (0 to 2 µM in 0.2 µM increments). FCCP injection solutions were prepared and loaded into the cartridge using the Bravo. (A) Kinetic graph of FCCP titration and relative OCR response. (B) Dose-response curve of FCCP titrations showing two independently performed experiments, N = 8 (8 rows × 1 plate per data trace). (C) % OCR, STDEV, and %CV values for all concentrations tested compared by day.

The consistency of potency determinations when replicates are generated across multiple microplates was also assessed. The Bravo was used to perform a 10-step, two-fold serial dilution series of AA (Figure 10A) and load the resulting solutions into the XFe96 sensor cartridge ports "A". Each serial dilution was performed independently i.e. a source plate containing eight stock wells of AA were diluted in parallel using eight different tips of the Bravo, yielding independent dilution series AA1 to AA8.

An XF assay was performed: three basal measurements were recorded, AA injected, then three further rate measurements were recorded (Figure 10B). The response was calculated as the ratio of the rate at measurement seven to the rate at measurement three (defined as 100%) and plotted as %OCR versus µM AA to generate IC_{50} values for each dilution series (Figure 10C). Results indicate that responses across the individual AA series are consistent for samples prepared by the Bravo, with the mean IC_{50} values in close agreement both within and across plates/days.

Overall, these data from model dose-response assays illustrate that the Bravo platform can prepare complex dilution series of compounds reproducibly. This reproducibility increases the likelihood that a single replicate plate provides a reliable response, thus allowing the data from dose-response assays to be combined across several replicate microplates. As noted previously, manual dilution can enhance the risks of small differences in volume transfer (which can be propagated throughout dilution steps), as well as the increased chance of cross-contamination of compounds. The Bravo ensures that small volumes are transferred accurately and precisely, with no cross-contamination of samples.

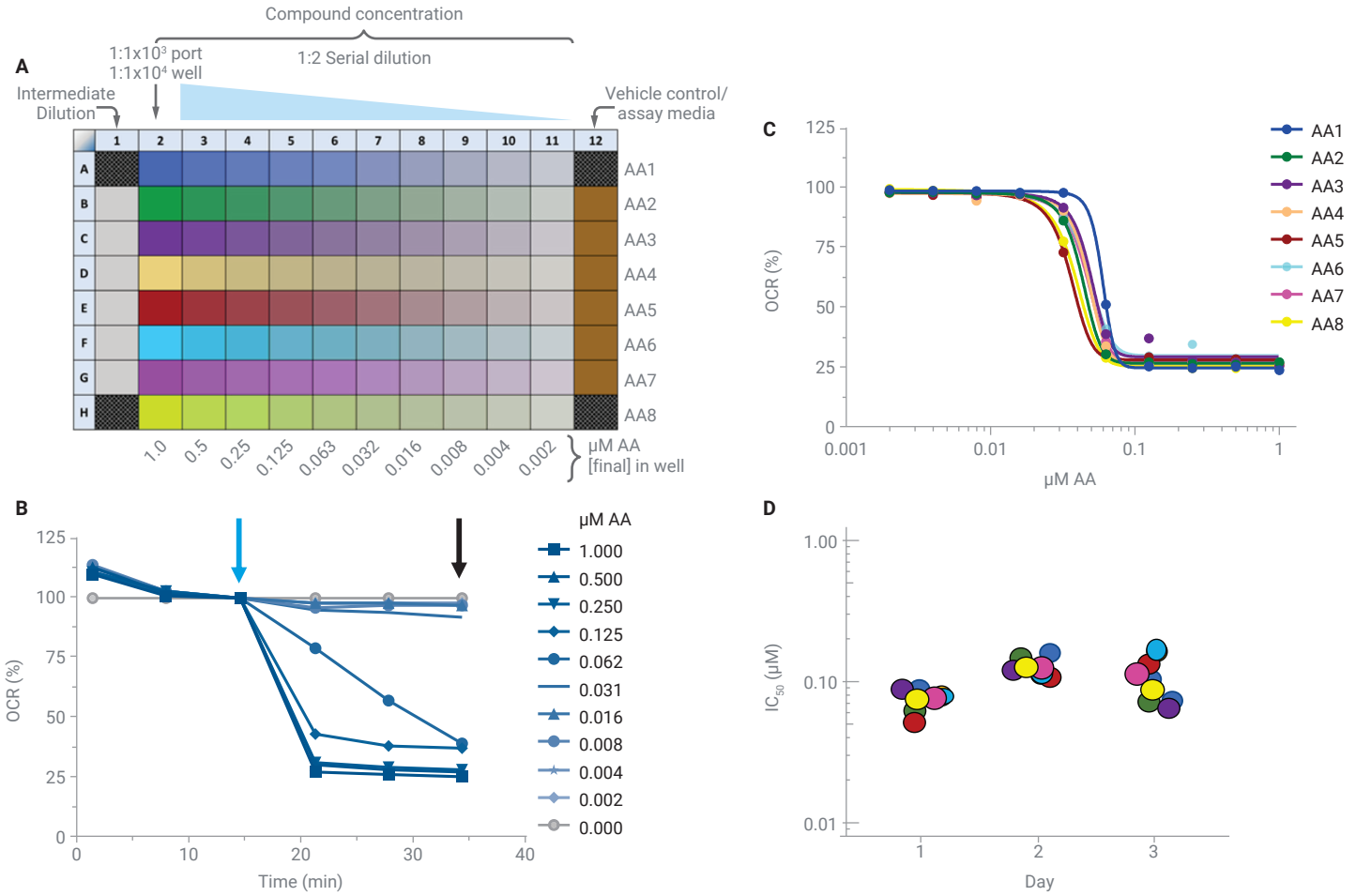


Figure 10. Compound dose-response assay. (A) Eight individually prepared dilution series of AA were labeled as AA1, AA2…AA8. Each unique series consists of a 10 step, two-fold serial dilution for final AA concentrations of 0.002 to 1.0 µM. Final concentration is shown across the bottom of the plate. (B) Resulting XF kinetic data for a single dilution series (AA1). (C) Resulting dose-response curves (AA1 to AA8) from a single plate. (D) Antimycin-A titration performance. IC_{50} values were calculated from each row, per the assay layout in panel A. Each dot represents an IC_{50} value calculated from a single dilution series (plate row). Values are consistent from row-to-row and day-to-day.

Finally, the Bravo can load XF cartridge ports in a single automated step rather than the multiple steps required by a manual operator, further decreasing associated variation or error associated with the latter.

Compound library screen – acute inhibition assay

A small compound library screen for mitochondrial and glycolytic inhibition was performed to illustrate the complete automated workflow. Seventy-two compounds from the Selleck-Pfizer L2400 library (Figure 11A) were screened for mitochondrial and glycolytic inhibitory activity. Bravo diluted the test compounds such that the final well concentration would be 10 µM and loaded the test compounds and controls into the injection ports.

In the Seahorse XFe96 Analyzer, basal rates were established in HepG2 as before, then library compounds were injected, and acute OCR and ECAR responses were recorded for 1 hour (Figures 11B and 11C). Responses were calculated as the ratio of the rate at 80 minutes (blue arrow) to the rate just before compound injection (orange arrow) and are expressed as % of the rate before compound injection. This assay was repeated three times each day for three days to generate nine replicate screening plates.

Z' is a standard measure of assay performance and thus was calculated for both primary outputs on each replicate plate (Table 2). For mitochondrial inhibition, the AA/Rtn compound cocktail is used as the positive control. For inhibition of glycolysis, 2-deoxyglucose (2-DG) was the positive control. The average Z' values for the mitochondrial inhibition assay was 0.88 and glycolysis inhibition assay was 0.77, indicating robust assay design and execution.

Table 2. Z' values for replicate Acute Inhibition Assays. OCR inhibition control (rotenone/AA) and ECAR inhibition control (2-DG) Z' values for three replicate assays performed over three days (n = 9 per compound).

Day-Plate	OCR Z'	ECAR Z'
1-A	0.92	0.84
2-A	0.87	0.72
3-A	0.83	0.29
1-B	0.89	0.87
2-B	0.88	0.86
3-B	0.88	0.84
1-C	0.88	0.84
2-C	0.86	0.77
3-C	0.90	0.88
AVERAGE Z'	**0.88**	**0.77**

A

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	AM	2-DG	BKG
B	2	10	18	26	34	42	50	58	66	AM	2-DG	BKG
C	3	11	19	27	35	43	51	59	67	AM	2-DG	No cells
D	4	12	20	28	36	44	52	60	68	AM	2-DG	No cells
E	5	13	21	29	37	45	53	61	69	VEH	AA/ Rot	No cells
F	6	14	22	30	38	46	54	62	70	VEH	AA/ Rot	No cells
G	7	15	23	31	39	47	55	63	71	VEH	AA/ Rot	BKG
H	8	16	24	32	40	48	56	64	72	VEH	AA/ Rot	BKG

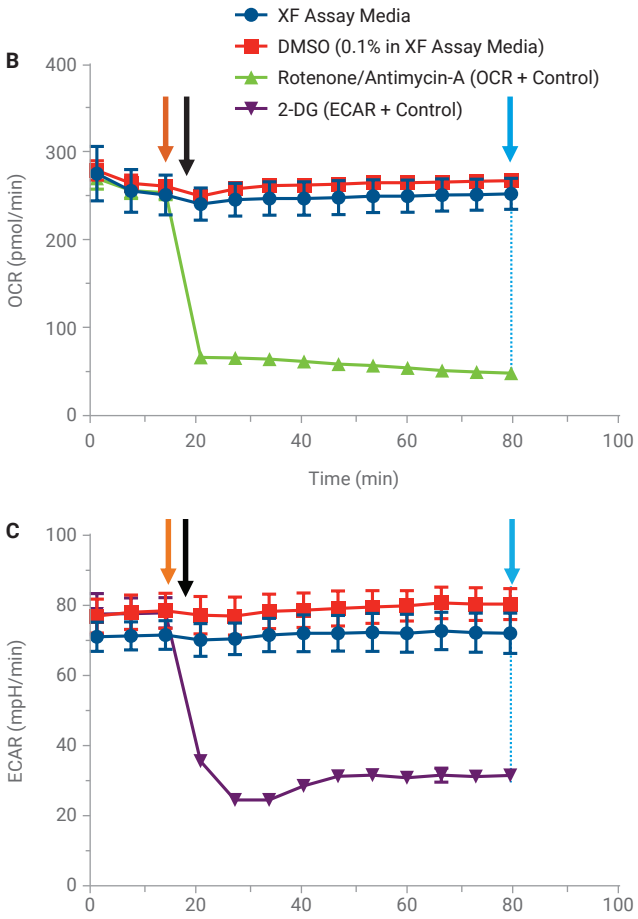


Figure 11. Compound library screen. (A) XFe96 plate map. Compounds are labeled by number (1 to 72), AM: assay media; VEH: DMSO control; 2-DG: ECAR positive control; Rtn/AA: OCR positive control. Representation of the XF assay for OCR (B) and ECAR (C), showing time of injection (black arrows). Data points used for calculation of % responses are indicated using orange (100%) and blue arrows, respectively.

For the compound library, responses were plotted as relative (%) OCR or ECAR (Figure 12). As evidenced by the low error bars representing SD of response for each compound, the inhibition assays are highly reproducible from plate to plate and day to day. This reproducibility and the Z' data indicate that using Bravo liquid handling automation and the Seahorse XFe96 platform is a robust method for detecting mitochondrial and glycolytic inhibitors.

In the assay design illustrated in Figure 11, cells were assessed before and 1 hour after compound injection within the same well. This platform also supports the design and performance of assays in which cells are treated with compounds before XF analysis (chronic exposure), to access longer-term kinetic effects of compound treatments. In this case, the Bravo would be used for dispensing of compounds directly into the XF cell culture microplate containing the cells of interest at predetermined times before the XF assay (e.g., 24 hours).

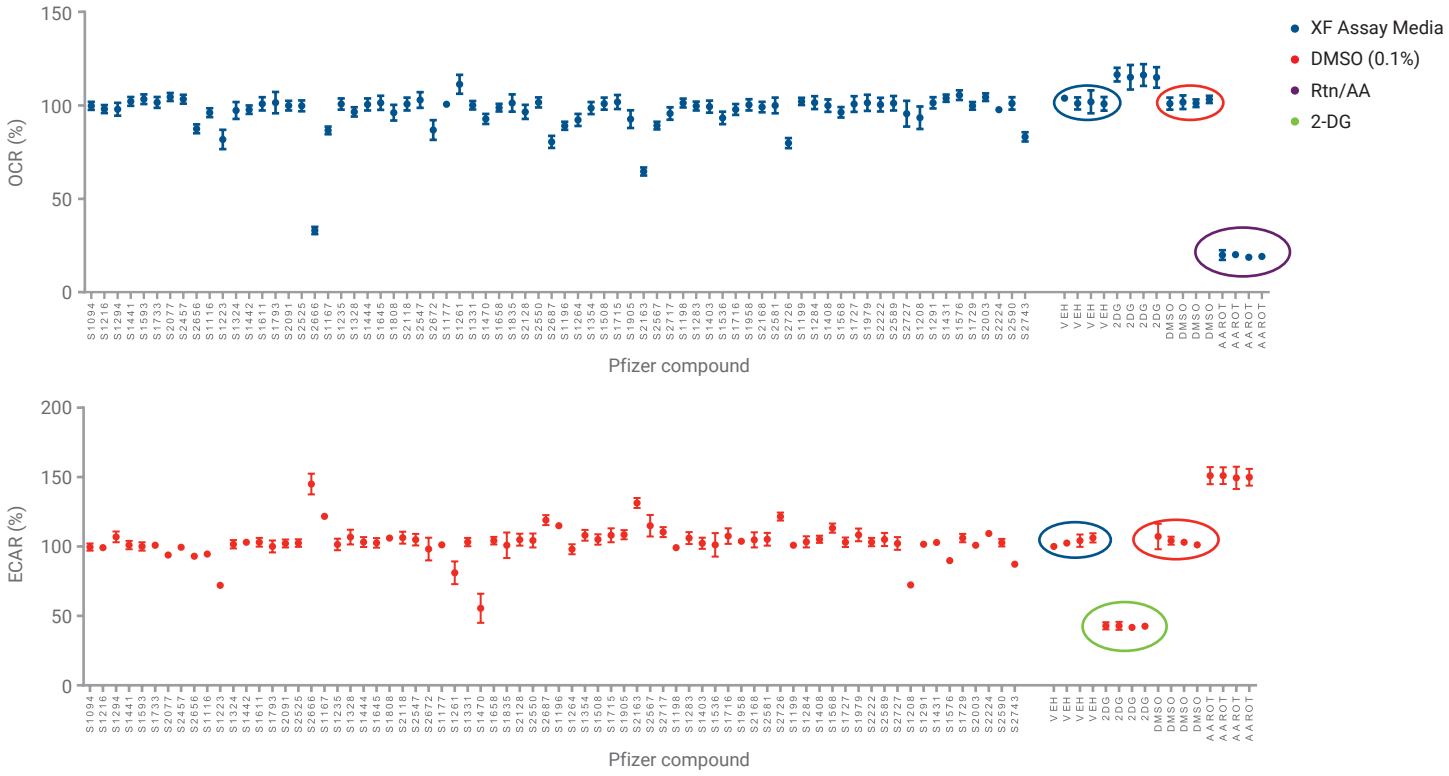


Figure 12. Example results of the acute inhibition assay using the Selleck-Pfizer L2400 library compound library. Responses for mitochondrial activity (% OCR) are shown in the top and glycolysis (% ECAR) at the bottom. Responses of positive control compounds rotenone/antimycin-A (OCR inhibitor, purple) and 2-DG (ECAR inhibitor, green), are shown as well. OCR and ECAR responses to vehicle control (0.1% DMSO) are circled in red.

Conclusion

This application note illustrates the methods and performance of automated liquid handling for XF assay sample preparation. Use of the Bravo platform to automate the steps of cell washing, injection solution preparation, and sensor cartridge port loading provided accurate and consistent results. The Bravo platform also provided the added benefits of: 1) reducing manual errors associated with repeated manual pipetting steps, 2) increasing consistency and reliability of data quality within and across microplates, and 3) decreasing manual "hands-on" time for XF assay preparation. Automation of key manual liquid handling steps of an XF assay thus results in greater efficiency of the XF assay workflow, as well as improved quality of resulting data.

Appendix

Table A1. Agilent Bravo automated liquid handling platform specifications for use with Agilent Seahorse XFe96 and XF Pro Analyzers (all heights measured relative to the bottom of the specified plate or surface).

Aspirate Height in Cell Plate	3 mm
Aspirate Speed for:	
XF96 Cell Plate	Fixed tip 51 to 200 µL
Reservoir (86 mL)	Default
Storage Plate (96)	Default
24-Column Reservoir	Default
Dispense Height for:	
Reservoir (86 mL)	2 mm
XFe96 Cartridge	0.5 mm
Dispense Speed for:	
XF96 Cell Plate	Fixed tip slow wash
Reservoir (86 mL)	Default
XFe96 Cartridge	384 disposable tip 10 to 50 µL

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