

Reagent Dispensing with the Formulatrix Tempest in a High-Throughput Screening Lab

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INTRODUCTION

In support of its SelectScreen® Services business, global biotech company Life Technologies researched and tested automated liquid handlers to streamline the addition of adenosine triphosphate (ATP) to Z'-LYTE® 384 well assay plates. Rigorous testing proved that the Formulatrix Tempest met all of Life Technologies's strict reliability, accuracy and precision requirements. Today, the Tempest is a routine part of Life Technologies's workflow.

ABOUT LIFE TECHNOLOGIES

Life Technologies is a global biotechnology company that offers world-class products and services to the diverse community of life-science professionals. These systems, consumables, and services enable researchers to accelerate scientific exploration, facilitating discoveries and developments that improve the lives of people everywhere.

Life Technologies's SelectScreen® Services (www.lifetechnologies.com/discoveryervices) provide high-throughput screening and compound profiling services to drug discovery clients. A diverse portfolio of biochemical and cellular assay formats is available for kinases, G protein-coupled receptors (GPCRs), nuclear receptors, ion channels, P450s, and human Ether-à-go-go-Related Gene (hERG) screening. Customers who have specific unmet needs can partner with the SelectScreen® Services team for custom assay development and screening. More than 17,000 projects have been completed since 2004, with >99.5% delivered on time.

The SelectScreen® Services team is passionate about delivering high-quality screening data while meeting client timelines. To meet these requirements, the team employs strict quality controls and automated processes in all aspects of these services. It is imperative that the equipment used by the team provides high accuracy and precision, as well as

flexibility, when dispensing reagents simultaneously during automated processing based on barcode-specific work lists.

In 2012, the SelectScreen® team set out to purchase a new piece of equipment to streamline ATP addition to 384 well assay plates. This new instrument needed to meet the following stringent requirements:

- Non-contact dispensing, resulting in no carryover between samples.
- Low volume dispensing, with 2.4 uL to 5.0 uL dispenses.
- High throughput capabilities, with the ability to process >50 384-well assay plates per hour.
- Capability to dispense up to 11 different reagents into unique/discrete plate locations.
- Ability to read plate barcode information to retrieve plate-specific dispense lists and dispense appropriately.
- Maximum ease of use for end users.
- Robust instrument performance/rapid maintenance and repair capability.
- Low dead volume for all loaded reagents.

Based on these requirements, the Formulatrix Tempest liquid handler was chosen for evaluation.



ABOUT THE TEMPEST

The Formulatrix Tempest is a non-contact, bulk reagent dispenser based on proprietary modular microfluidic chip technology. It is configurable to simultaneously deliver any volume of up to 12 ingredients through 96 individually controlled channels. The Tempest's patented microfluidic valve clusters use positive displacement to dispense discrete volumes of liquid to 96-, 384-, or 1536-well plates with non-recoverable dead volumes as low as 50 μ L per ingredient. The optional stackers and barcode reader add additional flexibility to the instrument.

PERFORMANCE TESTS AND RESULTS

Before new equipment is purchased and used in daily screening activities, it must pass thorough functional and non-functional testing. The following are some of the functional tests performed to evaluate the Formulatrix Tempest, along with the actual results that led to the purchase of the instrument and its subsequent routine use.

I. ACCURACY AND PRECISIONS TESTS

Any equipment used in the SelectScreen[®] lab must have very high repeatability and reproducibility. The team requires >95% accuracy when dispensing 2.4 μ L buffer and >90% precision on 2.4 μ L buffer dispenses for all installed chips.

For these tests, the Life Technologies 1x Kinase Buffer A was mixed with 10nM Fluorescein as the dispensing reagent. 2.4 μ L of 10nM Fluorescein was dispensed to plates with the Tempest, and backfilled manually with 12.6 μ L Kinase buffer A. The plates were shaken, spun down at 1000 RPM for one minute, and incubated for an hour. The accuracy was

determined gravimetrically, and the precision (%CV) was determined using a Tecan Safire2[™] fluorescence plate reader at 485/520 nm excitation/emission wavelengths. The results of these tests were well within the required values, as shown in Table 1.

II. CARRYOVER TEST

It is imperative for assay integrity that minimal / no carryover occurs between dispensing. To test for this, test plates were filled with 20nL 1mM Fluorescein in 100% DMSO in odd-numbered columns using a Labcyte Echo acoustic dispenser. The Tempest then dispensed Kinase buffer A to all wells, including empty and Fluorescein-containing wells, to a final volume of 15 μ L. The Tempest also dispensed 15 μ L buffer to an empty blank buffer plate. The plates were shaken, spun down at 1000 RPM for one minute, and incubated for an hour. The plates were read on a Tecan Safire2[™] plate reader at 485/520 wavelengths with a fixed gain for all plates. The fluorescent values of the buffer only wells on the test plates were analyzed and compared to the values of the blank buffer plate.

As Table 2 shows, the buffer-only wells on the blank plates and test plates had comparable standard deviations, precision, and values of fluorescence (RFU).

Additionally, direct dilution dispensing was tested by running 12 point standard curves using the Tempest to dispense Fluorescein as a direct titration followed by Kinase buffer for a final volume of 15 μ L. The 1.5x dilution had a starting concentration of 10 μ M Fluorescein in Kinase buffer. The plates were shaken, spun down at 1000 RPM for one minute, and incubated for an hour. The plates were read on a Tecan Safire2[™] plate reader at 485/520 nm excitation/emission wavelengths. The Tempest was able to achieve an average dilution of 1.49x, or 99% accurate titrations.

Tempest Chip Tested	Accuracy	Precision
Chip 1	99.6%	96.4%
Chip 2	96.7%	95.4%
Chip 3	99.2%	96.4%
Chip 4	98.3%	97.0%
Chip 5	98.3%	96.5%
Chip 6	98.3%	97.4%
Chip 7	98.8%	97.9%
Chip 8	97.1%	97.9%
Chip 9	97.9%	96.2%
Chip 10	99.2%	96.6%
Chip 11	99.2%	97.5%
Chip 12	99.6%	97.9%

Table 1. Accuracy and Precision Tests for the Tempest.

	Average RFU	Standard Deviation	%CV
Blank Plate 1	29923.8	1041	3.5%
Blank Plate 2	29275	1057.6	3.6%
Test Plate 1 Blank Columns	33238.3	1022.7	3.1%
Test Plate 2 Blank Columns	34312.5	1275.4	3.7%

Table 2. Carryover tests.

III. FUNCTIONAL TEST, ATP DISPENSES

In order to test ATP dispense performance on the Tempest, the Tempest was tested in a side-by-side comparison to manual ATP addition. Twenty assays were run using all 11 ATP reagent concentrations. The percent inhibition values acquired using automated dispensing compared favorably to those from manual addition, indicating that the Tempest is highly useful for ATP addition to Z'LYTE® assay plates (Figure 1).

TEMPEST IMPLEMENTATION

The SelectScreen® kinase profiling team frequently uses the Tempest to dispense ATP into Z'LYTE® assay plates. The Z'LYTE® biochemical assay employs a fluorescence-based, coupled-enzyme format and is based on the differential sensitivity of phosphorylated and non-phosphorylated peptides to proteolytic cleavage. ATP is the source of energy for this assay, and the amount of ATP in the reaction has

an effect on the 'activity' of the kinase or the amount of phosphorylation of the substrate.

SelectScreen® biochemical profiling services offer activity-based assays for >275 kinases, offered at 10 µM ATP, 100 µM ATP and Km apparent. This requires eleven separate reagent solutions of varying ATP concentration ranging from 5 µM to 500 µM. The team requires an instrument that has dynamic reagent selection in order to source the appropriate reagent for each assay being conducted according to the provided work list.

Because the concentration of ATP dispensed is critical to assay integrity, the method of delivering ATP must be very precise and accurate. The plate layout for each assay plate is unique, so this step works best when automated to ensure that the correct ATP reagents are delivered to the proper wells with high confidence.

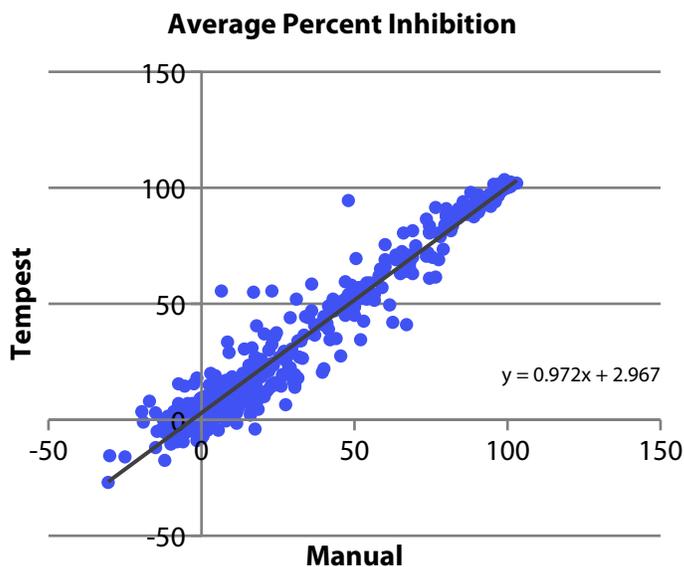


Figure 1. Percent inhibition comparison of manual vs. Tempest ATP addition to Z'LYTE assay

In routine production runs, the Tempest reads the barcode on the assay plate, which is linked to a file that lists the source ATP reagents and the destination wells for that specific plate. The Tempest then dispenses the various ATP reagents to the correct wells essentially simultaneously. The instrument also provides an audit trail of the plate dispenses and time dispensed.

SUMMARY

The SelectScreen® services are executed with stringent quality controls and automated processes to ensure reliable data is delivered every time. The Tempest has met the high standards that Life Technologies requires for automation used in the services, and is now an integral part of the routine workflow that has helped to drive efficiencies and increase throughput - ultimately allowing Life Technologies to deliver high quality data to its clients and even shorter timelines.