



Miniaturized Enzo Life Sciences HDAC1 Fluor de Lys Assays Using an Echo Liquid Handler Integrated in an Access Laboratory Workstation

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Abstract

Epigenetic targets continue to provide alluring opportunities for drug discovery efforts in the pharmaceutical industry. Such pursuits typically involve the investigation of a number of epigenetic enzymes, and screens for inhibitors of these targets could contribute to the rapid consumption of compound libraries in high-throughput campaigns. The acoustic dispensing technology utilized by the Echo 555 Liquid Handler enables nanoliter compound volume dispensing and has become the gold standard for delivering compounds for drug discovery efforts. The small volume dispensing of Echo Liquid Handlers has broader applications in drug discovery. The screening effort described in this application note utilized the 2.5 nL drop size capability of the Echo 555 Liquid Handler to dispense compounds and the larger volume 25 nL drop size capability of an integrated Echo 525 to dispense aqueous epigenetic reagents in an automated screening assay using the Access Laboratory Workstation.

Introduction

Epigenetics continues to emerge as an important target class for drug discovery and cancer research. Histone Deacetylase 1 (HDAC1) is an epigenetic enzyme that regulates gene transcription by removing acetyl groups from lysine residues on histones and causing chromatin condensation. Human cancers of the gastric system, prostate, colon and breast have demonstrated increased HDAC1 expression. As research programs scale to evaluate many new targets related to epigenetic function, new tools and techniques are required to enable efficient and reproducible high-throughput epigenetic screening. Enzo Life Science's Fluor de Lys assay technology is widely used for assessing the activity of epigenetic activity inhibitors. These assays are readily adaptable to high-throughput screening on the Access Workstation (Figure 1). In this study, the Echo liquid handling systems were used to miniaturize an HDAC1 Fluor de Lys assay to 3 μ L total volume in 384-well microplates for a 10 plate automated screening run. Prior to assembling assays, 25 nL of epigenetic library compounds and control inhibitors were transferred with the Echo 555 Liquid Handler to generate assay-ready plates. The assay-ready plates were then transferred to an Access workstation with an integrated Echo 525 Liquid Handler for automated, high throughput assay assembly. On the Access workstation, the Fluor de Lys assay reagents were delivered from the Echo Qualified Reservoir using the Echo 525 Liquid Handler (Figure 2). Employment of acoustic energy transfer enabled by Echo liquid handlers to generate assay-ready plates and deliver Fluor de Lys reagents resulted in a robust, automated and miniaturized assay that accurately identified known HDAC1 inhibitors.



Figure 1: The Access Laboratory Workstation

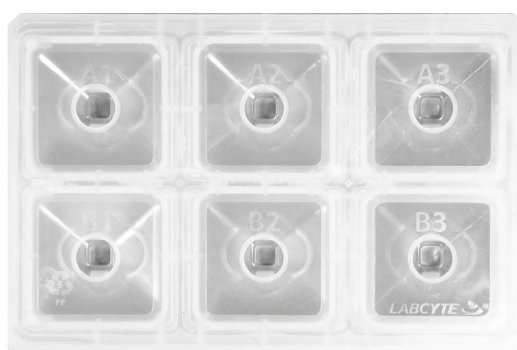


Figure 2: Echo Qualified Reservoir

HDAC1 Fluor de Lys Fluorogenic Assays

The HDAC1 Fluorescent Activity Assay/Drug Discovery Kit is a complete assay system designed to measure the deacetylase activity of recombinant human HDAC1. The deacetylase activity is assessed by measuring the conversion of fluorophore-labeled peptide substrate.

In the enzymatic reaction, the HDAC1 enzyme catalyzes the removal of the acetylated lysine side chain from the Fluor de Lys substrate. Deacetylation of the substrate sensitizes the substrate so that, in the detection step, treatment with the Fluor de Lys Developer produces a fluorophore (Figure 3).

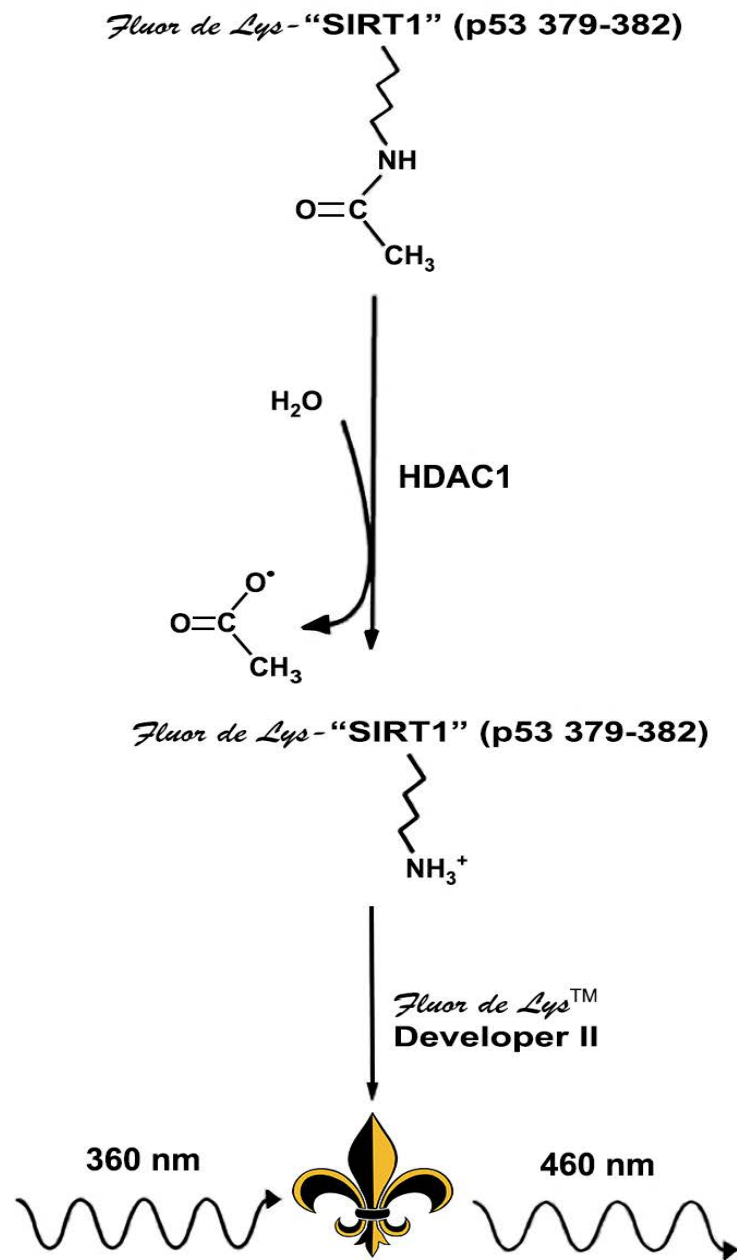


Figure 3: Enzo Life Science's Fluor de Lys Assay Technology

Diagram courtesy of Enzo Life Sciences, Inc.

Methods

HDAC1 Enzyme Titration: Experiment 1

The enzymatic activity of the HDAC1 enzyme was investigated in Enzo Life Science's Fluor de Lys Fluorogenic assay.

A 12-point, 1:3 serial titration of HDAC1 was prepared in the assay buffer provided in the Fluor de Lys kit. 1.25 μL of each dilution were transferred in triplicate along with 1.25 μL of 10 μM Fluor de Lys substrate from a 384-well Echo qualified polypropylene source plate to a 384-well assay plate using an Echo 525 liquid handler. The plate was then sealed and incubated at room temperature for 60 minutes. After incubation, 2.5 μL of the Trichostatin stop reagent /Developer II detection reagent cocktail was added from a 384-well Echo-qualified source plate to the assay plate using the Echo 525 liquid handler. The plate was re-sealed, and incubated for 60 minutes. After incubation, the plate was read on the PHERAstar FS and the Fluorescent signal was plotted versus log HDAC1 concentration (Figure 4).

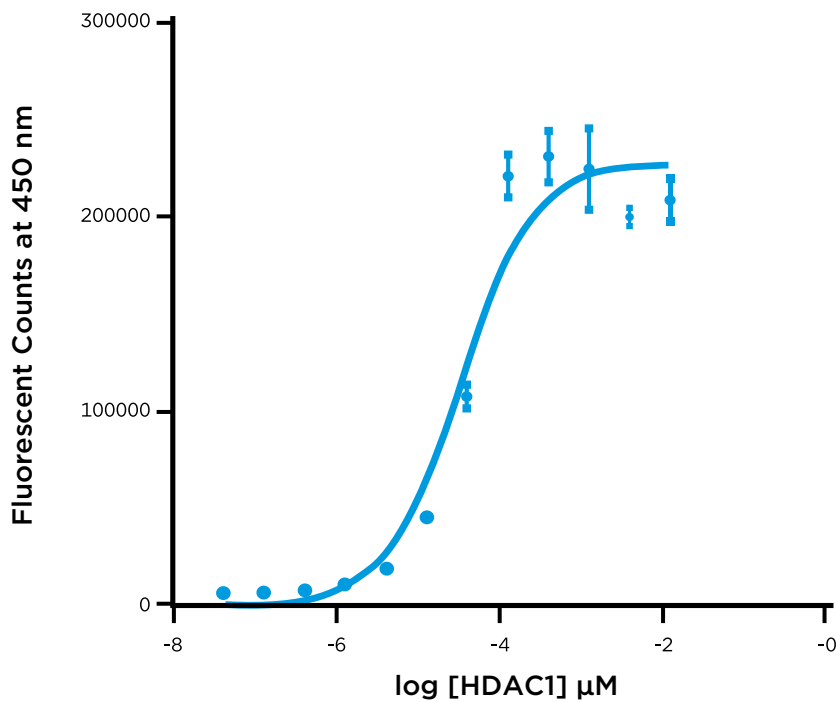


Figure 4: HDAC1 Enzymatic Titration

Automated HDAC1 Screening Run: Experiment 2

The SCREEN-WELL Epigenetic Compound Library from Enzo Life Science contains many known inhibitors of HDAC1. To determine the ability of the HDAC1 Fluor de Lys assay to accurately identify inhibitory compounds, the library was tested in an automated assay run using an Echo 525 Liquid Handler integrated onto the Access workstation.

Prior to initializing the automation run, ten assay-ready plates were prepared using an Echo 555 liquid handler : seven plates containing the Trichlostatin A (TSA) and CUDC-907 inhibitory control titrations as well as control wells representing the total and background signal of the assay and three plates containing 6 copies per plate of each library compound in 25 μM as well as the aforementioned inhibitory titrations and assay controls.

Total Volume	5 μL	2.4 μL
Compound / Buffer	50 nL	25 nL
Enzyme	1.2 μL	0.575 μL
Fluor de Lys substrate	1.25 μL	0.6 μL
Detection cocktail	2.5 μL	1.2 μL

Table 1: Assay reagent volumes

The assay ready plates were loaded onto the Access workstation. Using Tempo automation software, a schedule of tasks was executed to automate assembly of the assay: plates were delivered to the Echo system for addition of the enzymatic components of the assay (according to the volumes in Table 1) from an Echo qualified reservoir, then sealed, centrifuged and incubated at room temperature for one hour. The plates were then peeled and delivered to the Thermo fisher Multidrop Combi Reagent Dispenser for an addition

of the TSA stop reagent /Developer II detection reagent cocktail, and then sealed, centrifuged and incubated for one hour at room temperature before being read on the BMG Pherastar FS (see Figure 5 for the assay workflow). Percent Inhibition for the control and library compound values were calculated using the following formula: $((\text{Total signal} - \text{sample signal}) / (\text{Total signal} - \text{background signal})) * 100$.

For the control inhibitor titration, the percent inhibition values were plotted against the logarithm of the inhibitor concentration.

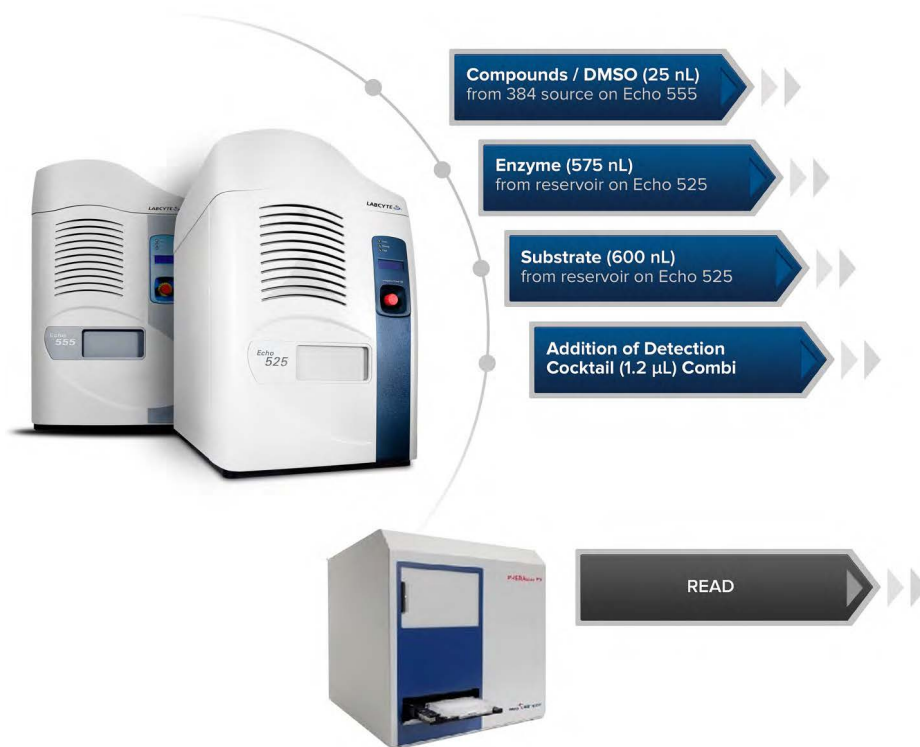


Figure 5: An HDAC1 Automated Workflow

Discussion

HDAC1 Titration: Experiment 1

An HDAC1 enzyme titration was performed using the Echo system for precise delivery of all of the assay components to a 384-well plate to reach a total volume of 5 uL. The HDAC 1 titration demonstrates a broad dynamic range and provides the user information about the proper enzyme concentration to use for future experiments. Development experiments like these are very straightforward to perform on the Echo system as all of the reagents were delivered with acoustic energy, resulting in a reduction of both hands-on-time and reagent consumption. The resulting data indicated that 40 nM HDAC1 would provide sufficient assay signal for the screening run.

Automated HDAC1 Compound Screen: Experiment 2

The ten plate HDAC1 screening run conducted on our Access Workstation with an integrated Echo 525 liquid handler produced high quality data and accurately identified HDAC1 inhibitors. The CUDC-907 and TSA control showed very consistent inhibition over the course of the run (Figure 6). The screening run had a representative Z' value of 0.72. Additionally, known HDAC1 inhibitors were identified by their strong percent inhibition values when tested at 25 μM. The consistency of the assay is also demonstrated by the small error bars in the percent inhibition determinations (Figure 7).

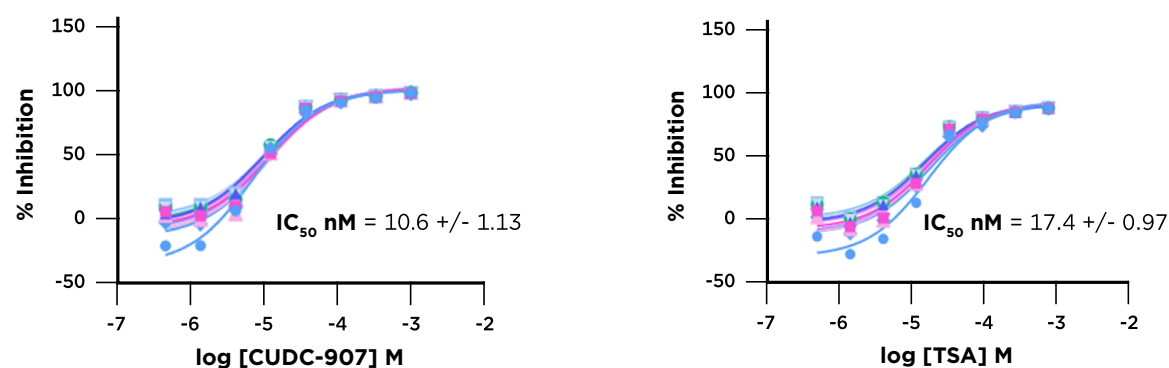


Figure 6: CUDC-907 and TSA Control Titrations

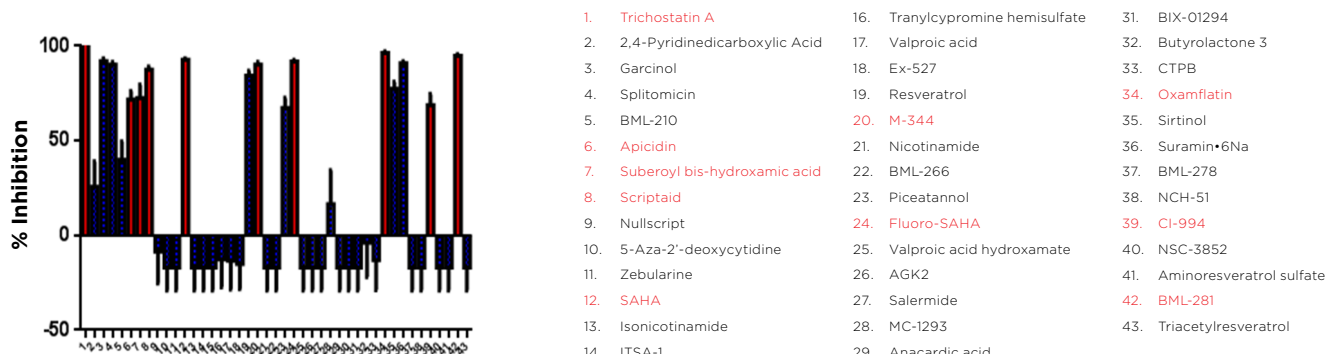


Figure 7: Percent Inhibition of SCREEN-WELL Library compounds

Summary

This workflow highlights the power and flexibility of acoustic liquid handling in the lab. The Echo 555 Liquid Handler was employed to rapidly and accurately dispense small volumes of compounds and controls to the assay-ready plates. The Access workstation with an integrated Echo 525 Liquid Handler completely automated this 10 plate screening run. The Echo Qualified Reservoir was used to deliver common reagents like HDAC1 enzyme and Fluor de Lys substrate to the assay plates accurately and with little volume wasted due to minimal dead volume required.

The employment of acoustic dispensing in this miniaturized assay run resulted in the accurate and efficient identification of known HDAC1 inhibitors with a cost and reagent consumption savings of over 80%.

Materials

Access Workstation Components

Component	Manufacturer
Access Workstation	Beckman Coulter Life Sciences
Echo 525 Liquid Handler	Beckman Coulter Life Sciences
Thermofisher Scientific Multidrop Combi Reagent Dispenser	Thermofisher
Agilent Technologies Microplate Centrifuge	Agilent
Agilent Technologies PlateLoc Thermal Microplate Sealer	Agilent
Brooks Life Sciences XPeel Microplate Peeler	Brooks

Assay Reagents

Assay	Manufacturer	Part Number
Human recombinant HDAC1	Enzo	BML-SE465-0050
FLUOR DE LYS HDAC1 fluorometric drug discovery assay kit	Enzo	BML-AK511-0001
Epigenetic SCREEN-WELL compound library	Enzo	BML-2836-0100
384-well Echo qualified polypropylene source plate	Beckman Coulter Life Sciences	001-14555
Echo Qualified Reservoir	Beckman Coulter Life Sciences	001-11101
384-well assay plate	Greiner	784075
Trichostatin A	Selleckchem	S1045
CUDC-907	Selleckchem	S2759

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AAG-7179APP06.20