APPLICATION NOTE

Miniaturization of an Epigenetic AlphaLISA Assay with the Echo Liquid Handler and the BMG LABTECH PHERAstar FS

Bonnie Edwards[1], Jing Wang[1], Carl Peters[2]

[1]Beckman Coulter Life Sciences, [2]BMG LABTECH

Abstract

Epigenetics continues to emerge as an important target class for drug discovery and cancer research. As programs scale to evaluate many new targets related to epigenetic expression, new tools and techniques are required to enable efficient and reproducible high-throughput epigenetic screening. The combination of robust and precise instrumentation from Beckman Coulter Life Sciences and BMG LABTECH enable the miniaturization and automation of PerkinElmer AlphaLISA epigenetic assay kits without compromising performance and data quality.

Background

Acoustic Dispensing and the Echo Liquid Handler

The Echo Liquid Handler revolutionizes liquid transfer by using acoustic energy to eject fluids. Echo Liquid Handlers can transfer compounds, samples, reagents, and beads in sub-microliter volumes to high-density assay formats using only acoustic energy – no contact or tips required, thereby reducing the risk of reagent carryover and eliminating tip costs.

Assay miniaturization increases screening throughput and reduces operating costs. With the ability to transfer fluids accurately and precisely in 2.5 nL and 25 nL increments, Echo Liquid Handlers provide a high-throughput liquid handling solution that enables miniaturization of screening assays without compromising data quality.

The PHERAstar FS Plate Reader

The PHERAstar FS multi-mode plate reader, with the highest sensitivity and lowest read time of assays in densities as high as 3456 wells, is a perfect complement to the Echo Liquid Handler. The PHERAstar FS plate reader uses a dedicated laser and assay specific modules for AlphaScreen / AlphaLISA detection that enables data collection at low assay volumes. Together the PHERAstar FS and Echo Liquid Handler offer an unparalleled solution for cost-effective, high-throughput epigenetic screening.

PerkinElmer AlphaLISA Assays

AlphaLISA assays are typically performed at volumes of about 20 to 25 µL. However, with the 2.5 nL and 25 nL dispense increment of Echo Liquid Handlers, assay volumes can be reduced significantly. In this study, we were able to reduce a methyltransferase assay volume to as low as a 5 µL with excellent results.

Experiment

AlphaLISA Methlytransferase Assay

We investigated the miniaturization of an AlphaLISA assay that measured the monomethylation of a biotinylated Histone H3 (1-21) peptide at Lysine 4 with the SET7/9 enzyme. In this assay, anti-methyl-Histone H3 Lysine 4 conjugated Acceptor beads bind specifically to the modified Histone H3 peptide. The Streptavidin Donor beads then bind to the biotinylated peptide, which brings them into close proximity to the bound Acceptor beads. Upon excitation of the Donor beads, singlet oxygen molecules are produced, resulting in a transfer of energy to the Acceptor beads, which then emit a signal at 615 nm.

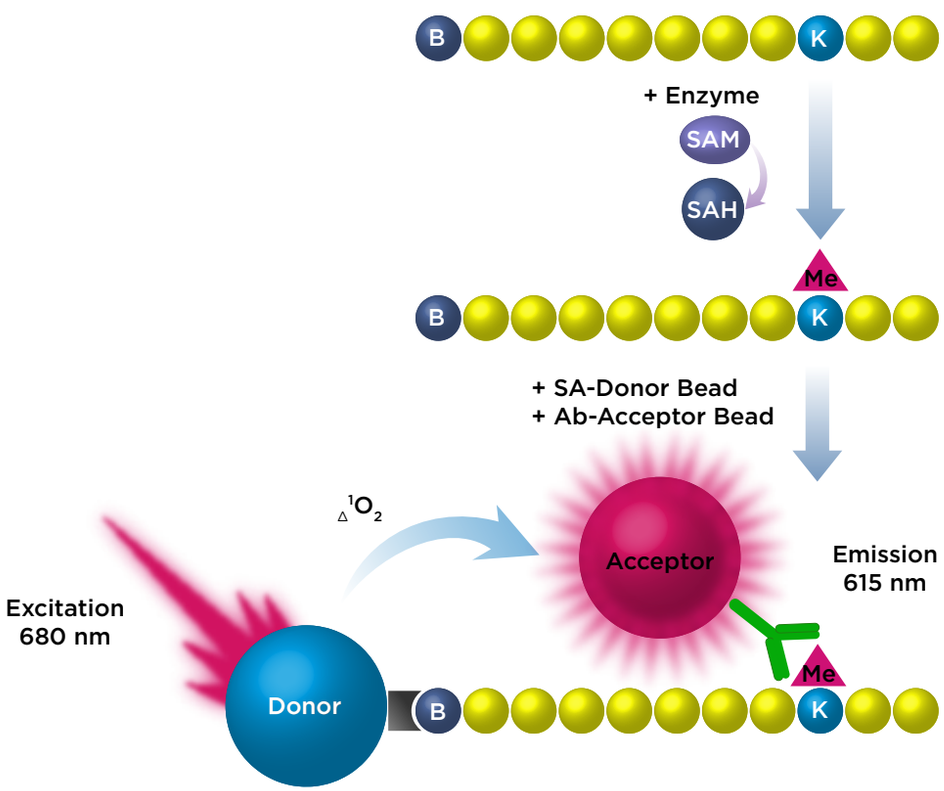

Figure 1: Detection of monomethylation of a peptide with AlphaLISA beads

Methods

A stock solution of S-(5'-adenosyl)-L-methionine chloride (SAM) was prepared at 30 mM in 5 mM H2SO4/10% ethanol (v/v) in H2O. Histone H3 peptide was diluted in assay buffer to a concentration of 200 nM (4X), SAM to 400 nM (4X), and SET7/9 enzyme to 4 nM (4X) just prior to use.

For both the 384-well and 1536-well assays, a dose response curve of two known inhibitors, S-(5'-adenosyl)-L-homocysteine (SAH) and sinefungin, were first added to a 384-well Echo Qualified Microplate source, and then transferred to the assay plates using the Echo 555 Liquid Handler. 40 nL of compound were transferred in the 10 µL assay and 20 nL of compound were transferred in the 5 µL assays.

Assay buffer, enzyme, and peptide + SAM (or peptide only for negative controls) were then added to the plates. In the 5 µL and 10 µL assays, the additions were performed using the Echo 525 Liquid Handler. The plates were sealed, briefly centrifuged at 1000 rpm, and incubated for 30 minutes at room temperature (RT).

Anti-methyl-Histone H3 Lysine 4 (H3K4me1-2) AlphaLISA Acceptor beads were diluted in 1X Epigenetics Buffer 1 at a concentration of 100 µg/mL, then added to a 384-well Echo Qualified Microplate source. Beads were then transferred to the assay plates with the Echo 525 Liquid Handler to a final concentration of 20 µg/mL. Plates were re-sealed, briefly centrifuged at 1000 rpm, shaken, and incubated for 60 minutes at RT, in the dark.

Streptavidin Donor beads were diluted in 1X Epigenetics Buffer 1 at a concentration of 50 µg/mL, added to a 384-well Echo Qualified Microplate source, and transferred to the assay plates with the Echo 525 Liquid Handler to a final concentration of 20 µg/mL. Plates were re-sealed, briefly centrifuged at 1000 rpm, shaken, and incubated for 30 minutes at RT, in the dark. Plates were then read on the PHERAstar FS plate reader.

As a comparison of signal to background (S/B) and Z' factors, positive and negative controls were also run at the standard assay volume of 25 µL in 384-well format. Reagent additions were done with manual pipetting.

Total Volume	**25 µL**	**10 µL**	**5 µL**
Assay Buffer	5 µL	2 µL	1 µL
Compound or DMSO	100 nL	40 nL	20 nL
SET7/9 Enzyme (4X)	2.5 µL	1 µL	500 nL
H3/SAM Mix (4X)	2.5 µL	1 µL	500 nL
Alpha Acceptor Beads	5 µL	2 µL	1 µL
Alpha Donor Beads	10 µL	4 µL	2 µL

Table 1: Volumes of reagents and sample added for each assay total volume

Methods were adapted from Gauthier, N, et al., Development of Homogeneous Nonradioactive Methyltransferase and Demethylase Assays Targeting Histone H3 Lysine 4, J Biomol Screen, 2012 17:49, online 21 September 2011

Results

We calculated signal to background and Z' factors for the different assay formats and compared the data for the miniaturized assay formats to the 25 µL assay format. While signal to background was lower in the miniaturized assays, overall assay quality in the miniaturized assay formats was still excellent, with Z' factors of 0.82 and above for all formats. In comparison, the 25 µL assay, which was assembled manually, had a Z' factor of 0.73.

Assay Format	Dispense Technique	S/B	Z' Factor
384-well, 25 µL	Manual	9.4	0.73
384-well, 10 µL	Echo System	6.6	0.82
384-well, 5 µL	Echo System	5.5	0.83
1536-well, 1 µL	Echo System	6.5	0.85

Table 2: Comparison of signal-to-background and Z' factors between standard volume assay and miniaturized assay formats

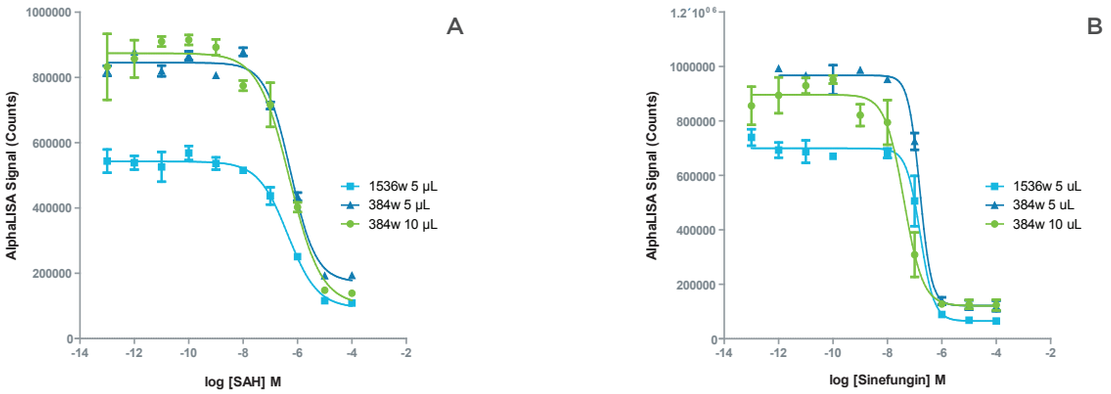

Figure 2: Inhibition curves for SAH and sinefungin showed good agreement in IC50 values between the miniaturized formats. A: Inhibition curves for SAH in miniaturized assay formats. B: Inhibition curves for sinefungin in miniaturized assay formats.

Assay Format	IC_{50} - SAH	IC_{50} - Sinefungin
384-well, 10 µL	515 nM	40 mM
384-well, 5 µL	566 nM	156 nM
1536-well, 5 µL	441 nM	161 nM

Table 3: Comparison of IC50 values for SAH and sinefungin in standard volume and miniaturized assay formats

Automation with the Access Laboratory Workstation

An epigenetic AlphaLISA assay can be readily adapted to automation with the Access Laboratory Workstation. The Access system allows seamless integration of an Echo Liquid Handler, including bulk fillers, plate sealers, plate peelers, centrifuges, and plate readers such as the PHERAstar FS. Tempo Automation Control Software manages all plate processing and has scheduling features to control plate incubation times. In this way, assay assembly can be automated from start to read on the Access system, thereby increasing throughput and maximizing walk-away time.

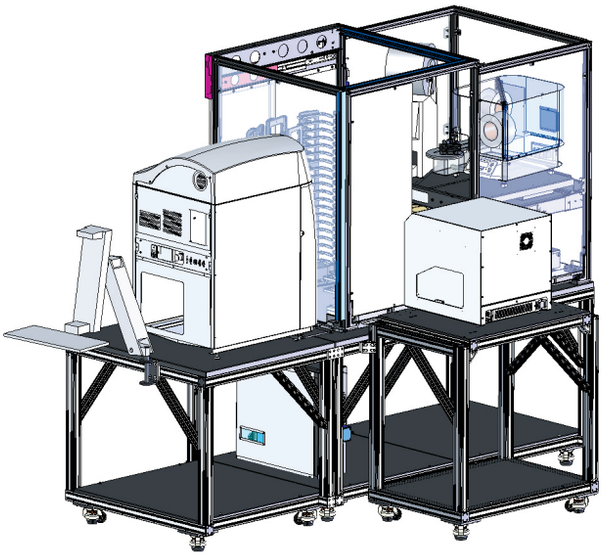

Figure 3: Access Laboratory Workstation featuring an Echo Liquid Handler and PHERAstar FS plate reader

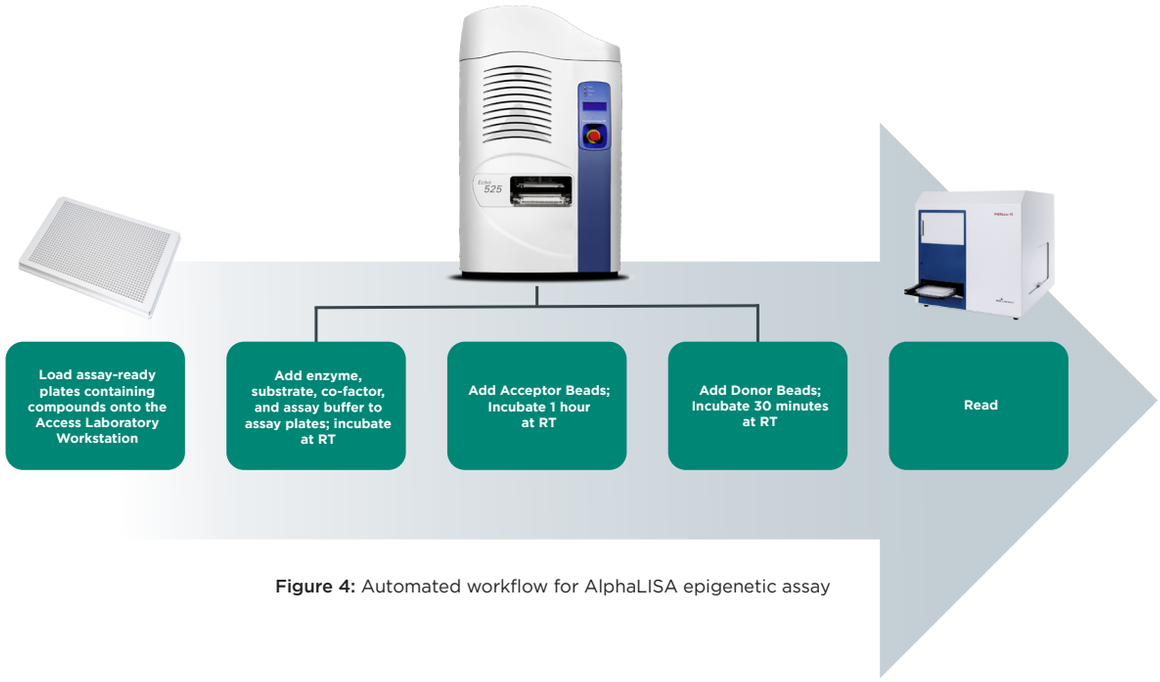

Figure 4: Automated workflow for AlphaLISA epigenetic assay

Summary

As epigenetics continues to grow as an area of focus, there is a greater need to be able to run these assays on a larger scale in a cost-effective manner. Doing so requires the ability to adapt these assays to miniaturization and higher-density formats. With the Echo Liquid Handler, reagents, and compounds can be dispensed accurately and precisely in nanoliter volumes, enabling the assembly of miniaturized AlphaLISA epigenetic assays while maintaining or improving data quality. Additionally, with the Access Laboratory Workstation, the entire workflow can be easily automated to increase throughput and productivity.

Materials

Equipment	Manufacturer
Echo 525 Liquid Handler	Beckman Coulter Life Sciences
Echo 555 Liquid Handler	Beckman Coulter Life Sciences
Microplate Centrifuge	Agilent Technologies
PlateLoc Thermal Microplate Sealer	Agilent Technologies
PHERAstar FS Plate Reader	BMG LABTECH
XPeel Automated Microplate Seal Removal	Brooks Automation
Teleshake	INHECO

Reagents	Manufacturer	Part Number
SET7/9 (human) Enzyme, Recombinant	Enzo Life Sciences	ALX-201-178-C100
Histone H3 (1 - 21) Peptide, Biotinylated	AnaSpec	AS-61702
Anti-methyl-Histone H3 Lysine 4 (H3K4me1-2) AlphaLISA Acceptor Beads	PerkinElmer	AL116
Alpha Streptavidin Donor Beads	PerkinElmer	6760002
AlphaLISA 5X Epigenetics Buffer 1 Kit	PerkinElmer	AL008
S-(5'-Adenosyl)-L-methionine chloride (SAM)	Sigma	A7007
S-(5'-Adenosyl)-L-homocysteine (SAH)	Sigma	A9384
Sinefungin	Enzo Life Sciences	ALX-380-070

Assay buffer: 50 mM Tris-HCl, pH 8.8, 5 mM MgCl2, 1 mM DTT, 0.01% Tween-20

Consumables	Manufacturer	Part Number
384-well Polypropylene Echo Qualified Microplate	Beckman Coulter Life Sciences	P-05525
384-well Round Bottom, White Microplate	Corning Life Sciences	3674
1536-well Flat Bottom, White Microplate	Corning Life Sciences	3729

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

AAG-6865APP05.20