dCypher™: Interrogating chromatin readers with high sensitivity and specificity

An assay to identify and assess reader binding to nucleosomes

Authors:
Matthew Marunde, Nathan Hall, Irina K. Popova, Matt Meiners, Jon Burg, Andrea L. Johnstone, Martis W. Cowles, Zu-Wen Sun, Michael-Christopher Keogh

EpiCypher Inc. Research Triangle Park, NC 27709
Correspondence: info@epicypher.com

Overview: Interrogating Chromatin Readers

Epigenetic reader proteins are of widespread interest for their essential roles in cellular function and the potential of targeted drug development to treat diseases such as cancer. Classical approaches rely on histone peptide pull downs or arrays to interrogate chromatin reader binding preferences[1]. However, these approaches are plagued by high failure rates due to poor sensitivity and/or high backgrounds. This is particularly problematic for resolving low affinity interactions, as is common for reader proteins with dissociation constants (Kd) in the micromolar range. Further, linear peptides cannot recapitulate *in vivo* interactions that occur in the context of three-dimensional nucleosome structure. To address these issues, EpiCypher has developed a sensitive and robust discovery platform to interrogate chromatin reader binding utilizing a diverse set of recombinant nucleosomes harboring distinct post-translational modifications (PTMs), histone variants, mutations, and/or DNA methylation states.

dCypher Chromatin Reader Assays: A homogenous biochemical assay to measure physiological chromatin interactions

This assay utilizes Perkin Elmer's AlphaScreen™ Technology, enabling a quantitative assessment of reader domain interactions with nucleosomes (Figure 1A). The homogenous "mix-and-measure" nature, absence of wash steps, high sensitivity and robustness of the approach make it ideal for interrogating low affinity chromatin reader-nucleosome interactions.

HP1ß is an established H3K9 methyl reader[2], binding the mono-, di- and tri-methyl states (Figure 1B). However, when presented with EpiCypher designer nucleosomes (dNucs), HP1ß displays markedly enhanced specificity for only H3K9me3 (Figure 1C). These findings emphasize the importance of studying epigenetic reader interactions in a biologically relevant nucleosome context.

The protocol described here allows end users to optimize a dCypher Chromatin Reader Assay by first using GST-tagged BRD4 Bromodomain 1 (BD-1) as a robust control. The protocol can then easily be adapted to accommodate a variety of epigenetic reader domains of interest, different protein tags (e.g. GST, His, or FLAG), and is compatible with EpiCypher's expanding biotinylated nucleosome portfolio. Further, this approach can be used to calculate Kd Apparents to more accurately compare similar reader and nucleosome binding pairs.

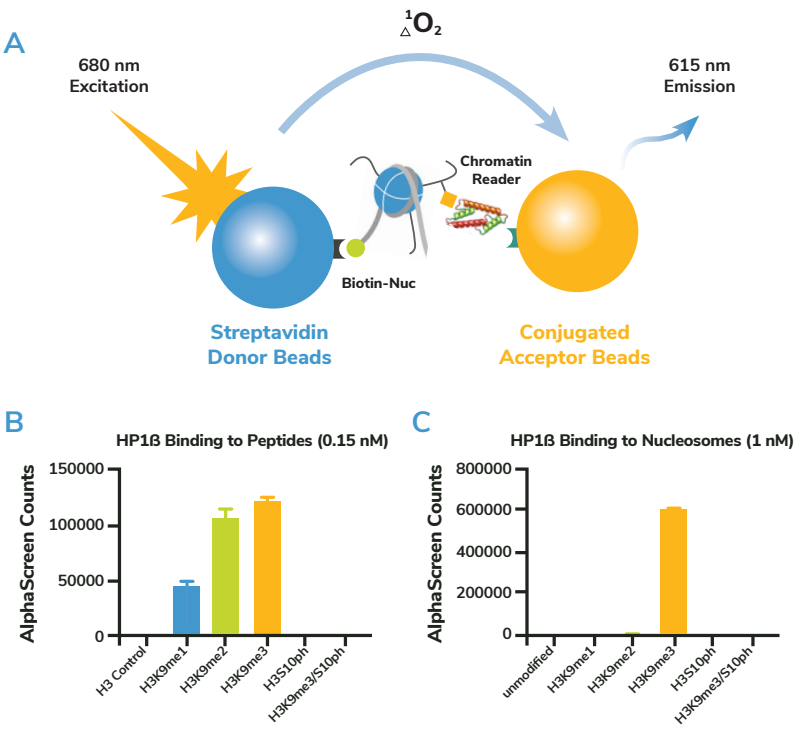

FIGURE 1: ASSAY PRINCIPLE. *The dCypher approach to interrogate chromatin readers.* (A) *Biotinylated nucleosomes are immobilized on Streptavidin Donor Beads and epitope-tagged chromatin reader is recognized by anti-tag conjugated Alpha Acceptor Beads. Laser excitation of the donor generates singlet oxygen that diffuses to activate emission from the acceptor. Fluorescence counts are directly proportional to the amount of donor-acceptor bridged by the nucleosome-reader interaction.* (B) *Binding of HP1ß (0.15 nM) to biotinylated histone peptides.* (C) *Binding of HP1ß (1 nM) to biotinylated nucleosomes.*

Continued...

EpiCypher®
Bringing Epigenetics to Life

dCypher: Interrogating chromatin readers with high sensitivity and specificity

TECHNICAL NOTE #111

An assay to identify and assess reader binding to nucleosomes

Continued from page 1

Reagents and Materials Required for the Assay:

NOTE: Store protein aliquots @ -80°C, avoid repeated freeze/thaw

REAGENT	REAGENT/MATERIALS	VENDOR	CATALOG#
Tagged reader protein *(e.g. His or GST)*	HP1ß Chromodomain (GST-tagged)	EpiCypher®	15-0058
	BRD4 Bromodomain 1 (GST-tagged)	EpiCypher®	15-0012
dCypher™ Nucleosome Panel *(e.g. full panel or focused sets)*	Full Panel	EpiCypher®	16-9001
	K-MetStat & OncoStat Panel	EpiCypher®	16-9002
	K-AcylStat Panel	EpiCypher®	16-9003
	R-MetStat Panel	EpiCypher®	16-9004
Detection reagents	Glutathione AlphaLISA Acceptor Beads	PerkinElmer	AL109M
	Nickel Chelate AlphaScreen Acceptor Beads	PerkinElmer	6760619
	AlphaScreen Streptavidin Donor Beads	PerkinElmer	6760002
Assay plates & seals	AlphaPlate-384, Light Gray	PerkinElmer	6005350
	TopSeal™-A Plus	PerkinElmer	6050185

Assay Buffer 1

20 mM Hepes pH 7.5, 250 mM NaCl, 0.01% BSA
add 0.01% NP-40 and 1 mM DTT fresh

Assay Buffer 2

20 mM Hepes pH 7.5, 250 mM NaCl, 0.01% BSA,
add 0.01% NP-40 fresh

Standard Protocol

NOTE: Briefly centrifuge plate (≤ 800 xg for ~10 seconds) after addition of each reagent to maximize reproducibility

*Do not vortex or sonicate nucleosome solutions

**To determine the optimal concentration of a reader protein of interest, first perform a titration with known positive and negative controls (e.g. Figure 2)

***AlphaScreen Donor beads are light sensitive and should be handled in subdued lighting.

****When utilizing Nickel Chelate Acceptor beads it is recommended to use 10 µg/mL SA Donor beads and 2.5 µg/mL Acceptor beads.

1. Add 5 µL of 4X nucleosomes* (10 nM final) prepared in assay buffer 1
2. Add 5 µL of 4X chromatin reader prepared in assay buffer 1**
3. Incubate 30 minutes at RT
4. Add 10 µL of 2X Streptavidin Donor Beads (5 µg/mL) and Glutathione Acceptor beads (2.5 µg/mL) in the DARK*** prepared in assay buffer 2****
5. Incubate 60 minutes at RT
6. Read using an Alpha Plate Reader: Ex 680 nm, Em 520-620 nm

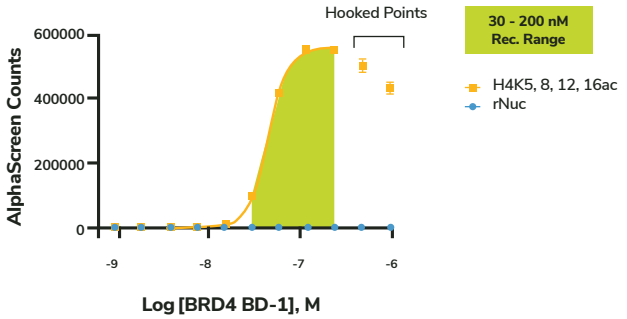

FIGURE 2: PROTEIN TITRATIONS. *Prior to testing a nucleosome library, the optimal screening concentration of the reader domain can be identified by performing a titration with a known target nucleosome and negative control. For BRD4 BD-1, binding to unmodified (rNuc, EpiCypher #16-0006) and tetra-acetylated (H4K5,8,12,16ac, EpiCypher #16-0313) nucleosomes was assessed. The ideal concentration range is 30 – 200 nM providing enough signal over background and below the hook point (the point at which signal begins to decease after saturation of the detection reagents). Each point is assayed in duplicate and presented as the mean +/- standard deviation.*

Q: *What do I do if my reader domain of interest has no known targets, or if I find that the known binding interactions discovered with histone peptides are not recapitulated on nucleosomes?*

A: *EpiCypher scientists have extensive experience with different classes of reader domains and experimental optimization.* Please contact us at info@epicypher.com to set up a science chat!

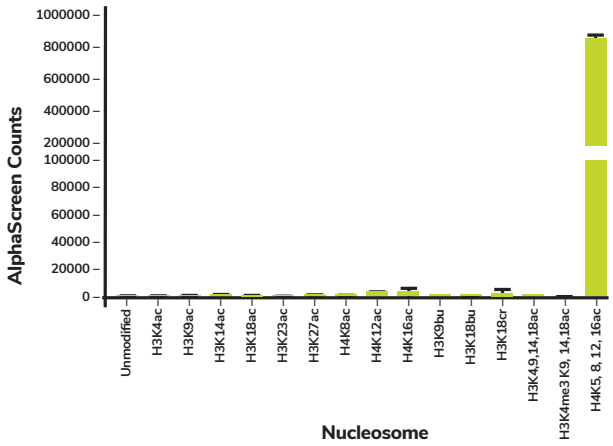

FIGURE 3: BRD4 BD-1 BINDING TO DCYPHER NUCLEOSOME K-ACYLSTAT PANEL. *EpiCypher has an ever expanding nucleosome library with individual and combinatorial modifications. BRD4 BD-1 binding to the dCypher K-AcylStat Panel was assessed using 100 nM protein (see Figure 2). BRD4 BD-1 displayed specificity for H4K5,8,12,16ac and minimal binding to individual acetyl modifications on H4 or acyl modifications on H3.*

References

1. Mauser R, Jeltsch A. Application of modified histone peptide arrays in chromatin research. Arch Biochem Biophys. 2019;661:31-38. doi: 10.1016/j.abb.2018.10.019.
2. Kim J, Daniel J, Espejo A, et al. Tudor, MBT and chromo domains gauge the degree of lysine methylation. EMBO Rep. 2006;7(4):397–403. doi:10.1038/sj.embor.7400625

EpiCypher®
Bringing Epigenetics to Life
855-374-2461 • info@epicypher.com • www.epicypher.com