EpiCypher® EpiDyne™ Chromatin Remodeling Platform User Guide

This User Guide describes EpiCypher's collection of EpiDyne™ nucleosome substrates and enzymes, and how these tools can be used to study ATP-dependent chromatin remodelers.

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1. Background on Chromatin Remodeling Complexes & Assays

Nucleosomes are the basic repeating unit of chromatin, consisting of 147 bp DNA wrapped around a histone protein octamer[3]. Nucleosome structure and positioning on DNA are regulated by ATP-dependent chromatin remodeling enzymes. These enzymes and associated protein complexes are actively involved in maintaining key genomic functions, including transcription, DNA replication, and DNA repair. Chromatin remodeling complexes are categorized into four main families (SWI/SNF, ISWI, CHD and INO80) based on their core ATPase structure, motor movement, and distinct nucleosome sliding or histone exchange mechanism (**Table 1**; see[4-6]).

Aberrant nucleosome organization and remodeler dysfunction are linked to diverse cancers and disease states (**Table 1**). Nearly 20% of all human cancers contain mutations in subunits from the SWI/SNF family of chromatin remodeling complexes, making them attractive therapeutic targets[7,8]. Recent studies have revealed remarkable structural and mechanistic information on other classes of chromatin remodelers and opened the doors for novel drug development[9-12].

Family	Mechanisms and Functions	Complex	ATPase(s)	Cryo-EM	Links to Disease
SWI/SNF	• Nucleosome sliding, ejection • Regulates accessibility and gene expression • Roles in DNA replication and repair	BAF	SMARCA2 /BRM OR SMARCA4/BRG1	See[11]	• Neurodevelopmental disorders (i.e. Coffin-Siris syndrome) • Many cancers; rhabdoid tumors, synovial sarcoma • See[13]
		PBAF	SMARCA4/BRG1	See[10]	
ISWI	• Nucleosome sliding, spacing • Supports nucleosome assembly and maturation • Limits accessibility to repress gene expression	NURF	SMARCA1/ SNF2L		• Neurodevelopmental disorders (e.g. microcephaly) • Many cancers (melanoma, lung, neuroblastoma, bladder) • See[14,15]
		ACF	SMARCA5/ SNF2H	See[16]	
		CHRAC			
CHD	• Nucleosome spacing, sliding, editing • ATPases uniquely contain chromodomains • Alters accessibility to regulate gene expression	NuRD	CHD3, CHD4	See[17]	• Neurodevelopmental disorders • Some cancers (neuroblastoma) • CHARGE syndrome • Dermatomyositis • See[18]
		CHD1	CHD1		
INO80	• Nucleosome sliding • Exchanges variant histones • Roles in DNA repair & transcription	INO80	INO80	See[19]	• Neurodevelopmental disorders (Floating-Harbor syndrome) • Some cancers (melanoma) • Congenital heart disease • See[20]
		TIP60	p400		
		SRCAP	SRCAP	See[21]	

Table 1. Summary of human chromatin remodeling complexes, their biochemical mechanisms and cellular functions[4], and associations with human disease. Note that not every complex or ATPase is captured here; see[4,22] for a review.

Chromatin Remodeling Assays: Historical Perspective

Research on chromatin remodeling enzymes comes with multiple challenges. Accurate studies demand highly pure, physiological nucleosome substrates, nucleosome-compatible workflows, and direct quantification of remodeling activity. These substantial requirements are made more challenging when chromatin remodelers are targeted for drug development, which necessitates lower cost, high-throughput screening (HTS) assays. HTS platforms are ideal for compound screening, since the homogeneous, no-wash assays provide greater sensitivity and throughput.

The most common assays used to study ATP-dependent chromatin remodeling enzymes are ATPase assays (e.g. radioactive ATPase assays, ADP-Glo, Phosphate Sensor), which measure the rate of ATP hydrolysis by detecting either ADP or inorganic phosphate. These assays are simple, compatible with nucleosome substrates, and suitable for HTS applications. Yet, ATPase assay platforms lack a central component of remodeling studies: they do not directly report on the nucleosome state, and instead provide an indirect measurement of enzyme activity. Results from these assays may be misleading, since ATPase activities can be uncoupled from chromatin remodeling in disease[23].

Scientists have developed other assays that can directly quantify repositioning of nucleosomes on DNA templates, including Restriction Enzyme Accessibility (REA) assays, Dam DNA methyltransferase-coupled radiometric assays, and Electrophoretic Mobility Shift Assays (EMSAs). However, these assays often require extensive optimization and large amounts of nucleosome substrate and enzyme[24,25]. They are also incompatible with HTS applications due to low throughput, reduced sensitivity, and relatively high cost per reaction. Furthermore, radiometric assays have increased safety concerns and infrastructure restrictions.

Thus, although these nucleosome-based assays have proved essential for understanding basic remodeling mechanisms, improved technologies are needed to push the boundaries for innovative therapeutic applications.

The EpiDyne™ Platform & Services for Chromatin Remodeling Research

EpiCypher's EpiDyne™ Chromatin Remodeling Platform & Services provides a unique solution to this problem. Our validated ATPase enzymes and nucleosome repositioning substrates are optimized for robust results using traditional assays (i.e. EpiDyne-REA) and HTS approaches (i.e. EpiDyne-FRET), making them compatible with basic research, drug discovery and mechanism-of-action studies. We have also developed a novel HTS-compatible platform, EpiDyne-PicoGreen, that uses ~75% less enzyme compared to other techniques (e.g. REA and FRET). See **Figure 1** for a summary of the EpiDyne approaches. Although our nucleosome substrates are suitable for ATPase and Dam DNA methylation assays these applications are not supported by EpiCypher, and information is not included in this User Guide.

In this User Guide we outline EpiDyne nucleosome substrates, enzymes, and their application for chromatin remodeling research (**Figure 1**). See Section 4.1 to learn more about which EpiDyne assay and nucleosome substrate is best for your research. For information about EpiDyne Assay Services, see epicypher.com/EpiDyne-Services.

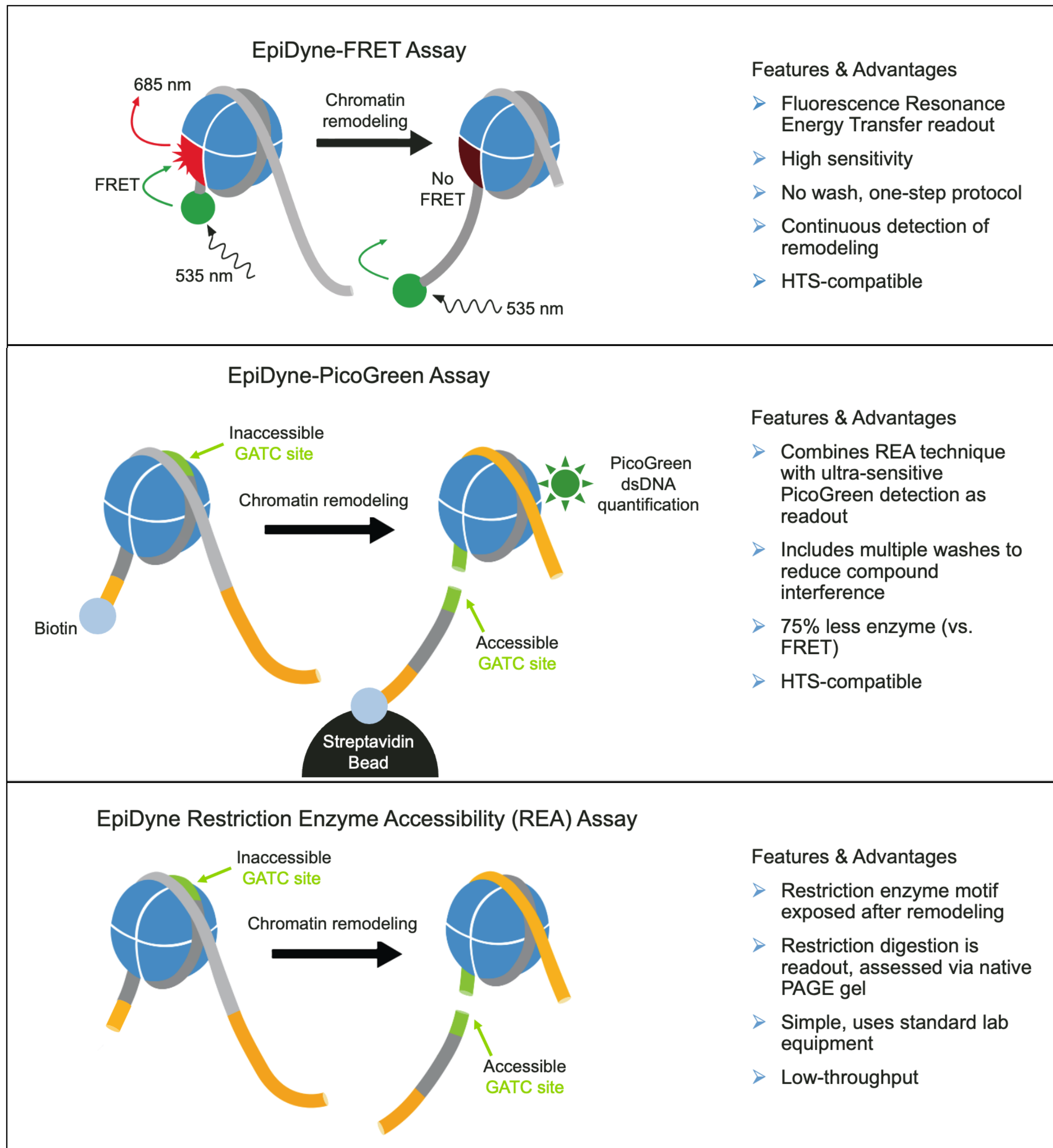


Figure 1. Review of EpiDyne Chromatin Remodeling Assays. Each assay provides a direct readout of nucleosome repositioning. Learn more about each assay in Section 4 of this Guide.

2. EpiDyne Chromatin Remodeling Enzymes

EpiCypher offers enzymes representing various ATPase families (**Table 2**). These enzymes carry out distinct remodeling functions and represent high-value drug targets.

EpiDyne Enzyme	Protein Family	Cat. No.	Species	Format	Protein Tag	Nucleosome Preference
SMARCA2/BRM	SWI/SNF	15-1015	Human	Full-length	6His-FLAG (C-terminal)	No preference
SMARCA4/BRG1	SWI/SNF	15-1014	Human	Full-length	6His-FLAG (C-terminal)	No preference
SMARCA5/SNF2H	ISWI	15-1024	Human	Full-length	FLAG-6His (N-terminal)	Terminally positioned
ACF complex	ISWI	15-1013	*Drosophila*	Acf1 + ISWI (Full-length)	6His-FLAG	Terminally positioned

Table 2: EpiDyne Chromatin Remodeling Enzymes available from EpiCypher. See epicypher.com/epidyne-enzymes for additional information.

2.1 SMARCA2/BRM and SMARCA4/BRG1

Background: SMARCA2 and SMARCA4 are mutually exclusive ATPases in SWI/SNF remodeling complexes with largely redundant functions. SWI/SNF remodelers play key roles in transcriptional regulation, genome replication and DNA repair[4], and mutations in SMARCA2 and SMARCA4 are found in many cancers (see Section 1, **Table 1**). Simultaneous mutation of SMARCA2 and SMARCA4 results in synthetic lethality, which is sufficient to arrest cancer cell growth and induce cell death[8,26]. SMARCA4 mutant cancer cells are often hypersensitive to SMARCA2 inactivation, making SMARCA2 an important target for therapeutic intervention[27].

Product Information & Applications: EpiCypher is the exclusive provider of enzymatically active, full-length human SMARCA2 (EpiCypher 15-1015) and SMARCA4 (EpiCypher 15-1014). Enzymes are expressed with a C-terminal 6His-FLAG tag. Both ATPases show robust activity in chromatin remodeling reactions, and do not distinguish between terminally and centrally positioned nucleosomes *in vitro* (in agreement with[28]; see Section 4.5).

Concentration of Enzyme for Assays: The unit definition of SMARCA2 and SMARCA4 enzymes is based on remodeling activity in EpiDyne-FRET (**Figure 2**, next page), and each product contains sufficient enzyme for 100 EpiDyne-FRET reactions. Enzyme concentration and recommended working concentration for EpiDyne-FRET assays is lot-specific; always refer to the TDS (see product pages, Documents and Resources). If applying to other formats (ATPase-Glo, etc.) we strongly recommend titrating the enzyme to determine ideal conditions for your assays.

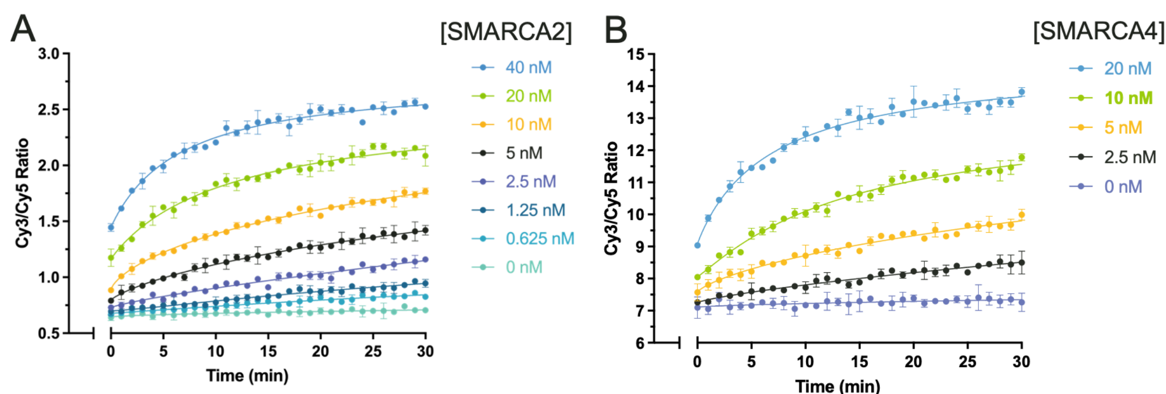


Figure 2. Example SMARCA2 and SMARCA4 validation data. EpiDyne-FRET nucleosome substrate (EpiCypher 16-4201; 20 nM) was incubated with varying amounts of SMARCA2 **(A)** or SMARCA4 **(B)** remodeling enzyme. Chromatin remodeling activity was determined by loss of FRET (i.e. increase in the ratio of Cy3 to Cy5 signal).

2.2 SMARCA5/SNF2H

Background: Human SMARCA5/SNF2H is the core ATPase subunit of multiple ISWI ATP-dependent chromatin complexes (see Section 1, **Table 1**). ISWI remodelers regulate nucleosome spacing to control DNA accessibility[4]. Structural and biochemical studies have revealed that SMARCA5 exhibits distinct substrate preferences and remodeling directionalities dependent on associated regulatory subunits[29]. Misregulation of SMARCA5 is linked with multiple human cancers, including leukemia, breast, lung, and gastric cancers, making it an attractive drug target[30].

Product Information & Applications: EpiCypher provides full-length, recombinant human SMARCA5/SNF2H containing an N-terminal FLAG-6His tag (EpiCypher 15-1024). The full-length SMARCA5 enzyme shows robust activity to reposition terminal nucleosomes in biochemical remodeling assays such as EpiDyne-FRET (**Figure 3**). SMARCA5 is highly sensitive to flanking DNA states on nucleosome substrates[29], with over 4-fold higher remodeling activity on terminally positioned nucleosomes compared to centrally positioned nucleosomes *in vitro* (see Section 4.5).

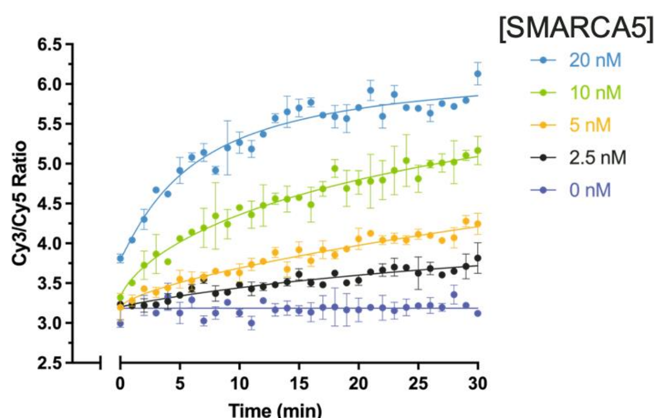


Figure 3. Example SMARCA5 validation data. EpiDyne-FRET nucleosome substrate (EpiCypher 16-4201; 20 nM) was incubated with varying amounts of SMARCA5 Remodeling Enzyme. Chromatin remodeling activity was determined by loss of FRET (i.e. increase in ratio of Cy3 to Cy5 signal).

Concentration of Enzyme for Assays: The unit definition of SMARCA5 is based on remodeling activity in EpiDyne-FRET (**Figure 3**), and each product contains sufficient enzyme for 100 EpiDyne-FRET reactions. Enzyme concentration and recommended working concentration for EpiDyne-FRET assays is lot-specific; always refer to the TDS (see epicypher.com/15-1024, Documents and Resources). If applying to other formats (ATPase-Glo, etc.) we strongly recommend titrating the enzyme to determine ideal conditions for your assays.

2.3 *Drosophila* ACF Remodeling Complex

Background: *Drosophila* ACF (ACF) belongs to the ISWI family of ATP-dependent chromatin remodeling complexes (see Section 1, **Table 1**) and comprises the core ATPase ISW1 and an accessory subunit ACF1. ISWI remodelers regulate nucleosome spacing to control DNA accessibility, regulate gene transcription and support genomic stability[4]. Of note, the ACF complex prefers terminally positioned nucleosome substrates, and displays minimal activity on central positioned nucleosomes[31,32].

Product Information & Applications: EpiCypher provides recombinant ACF at >90% purity (100 reactions; EpiCypher 15-1013). ACF possesses robust activity to reposition terminal nucleosomes in biochemical remodeling reactions such as EpiDyne-FRET (**Figure 4**; see Section 4.4). For more details, see the TDS at epicypher.com/15-1013.

Concentration of Enzyme for Assays: The unit definition of ACF is based on remodeling activity in EpiDyne-FRET (**Figure 4**), and each product contains sufficient enzyme for 100 EpiDyne-FRET reactions. Enzyme concentration and recommended working concentration for EpiDyne-FRET assays is lot-specific; always refer to the TDS (epicypher.com/15-1013, Documents and Resources). If applying to other formats (ATPase-Glo, etc.) we strongly recommend titrating the enzyme to determine ideal conditions for your assays.

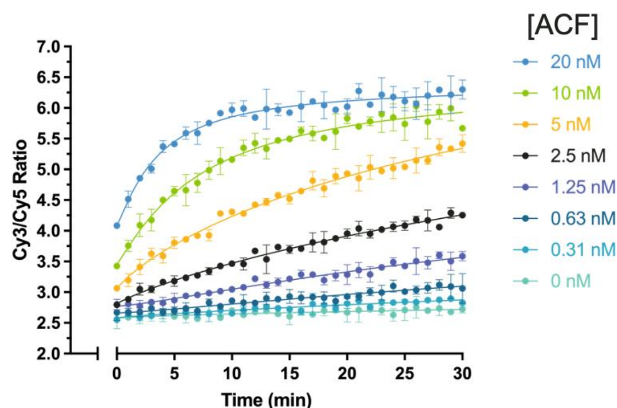


Figure 4. Example of ACF validation data. EpiDyne-FRET Chromatin Remodeling Substrate (EpiCypher 16-4201; 20 nM) incubated with ACF Remodeling Enzyme at varying concentrations. Chromatin remodeling activity was determined by loss of FRET (i.e. increase in ratio of Cy3 to Cy5 signal).

3. EpiDyne Remodeling Nucleosome Substrates

EpiCypher's EpiDyne™ Nucleosome Remodeling Substrates provide a diverse collection of chromatin remodeling substrates to maximize compatibility with different assays and enzymes (**Table 2**). Of note, EpiDyne substrates can be customized with histone post-translational modifications, embedded DNA motifs, or various flanking DNA lengths using our custom nucleosome development services (epicypher.com/services/custom-nucleosome-development).

Nucleosome Collection	Nucleosome Positioning	Cat. No.	GATCs	Tag	DNA control	Assay Compatibility		
						REA	FRET	PicoGreen
EpiDyne-FRET	5' Terminal (1-N-66)	16-4201	1	Cy3 (5'-DNA), Cy5 (H2AT120C)	18-4201	✓	✓	✗
EpiDyne-GATC Terminal	5' Terminal (6-N-66)	16-4101	1	None	18-4100 18-4101	✓	✗	✗
		16-4111	1	Biotin-TEG (5'-DNA)	18-4111 18-4110	✓	✗	✓
		16-4112	2	Biotin-TEG (5'-DNA)	18-4112	✓	✗	✓
		16-4113	3	Biotin-TEG (5'-DNA)	18-4113	✓	✗	✓
EpiDyne-GATC Central	Central (50-N-66)	16-4114	1	Biotin-TEG (5'-DNA)	18-4114	✓	✗	✓
		16-4115	2	Biotin-TEG (5'-DNA)	18-4115	✓	✗	✓
		16-4116	3	Biotin-TEG (5'-DNA)	18-4116	✓	✗	✓

Table 3: EpiDyne Chromatin Remodeling Nucleosome Substrates available from EpiCypher. See epicypher.com/EpiDyne-chromatin-remodeling-substrates for additional information.

3.1 EpiDyne FRET Nucleosome Remodeling Assay Substrate

Background: The EpiDyne-FRET Nucleosome Substrate (EpiCypher 16-4201) is specifically designed for homogeneous remodeling assays using Fluorescence Resonance Energy Transfer (FRET)[33]. The nucleosome comprises a human histone octamer containing an Cy5-tagged H2A and is wrapped by a Cy3-labelled DNA template (**Figure 5A**). The Widom 601 nucleosome positioning sequence[34] is located at the 5' end of the DNA template, resulting in a terminally-positioned nucleosome (**Figure 5B**). To reinforce terminal assembly, the 5' positioning sequence is flanked by TGGA repeats, which are refractory to nucleosome wrapping[34,35]. However, the 3' terminus also contains an acceptor sequence to accommodate the histone octamer after remodeling (not shown). A schematic of the DNA template is provided in **Figure 5B**, where **N** refers to **N**ucleosome positioning sequence; there is 1 bp upstream and 66 bp downstream of the final positioned nucleosome (1-**N**-66).

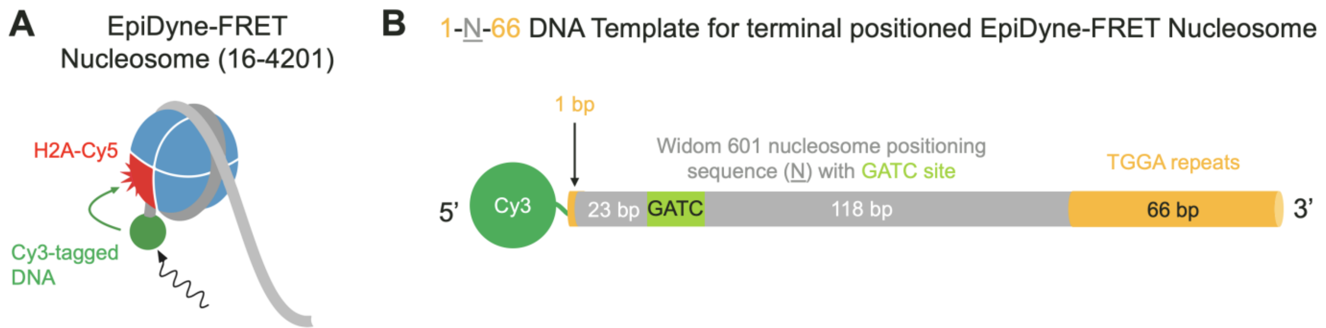


Figure 5. (A) EpiDyne FRET nucleosome (EpiCypher 16-4201). The nucleosome consists of a Cy5-labeled human histone octamer (H2A-Cy5; red section of octamer) wrapped by a DNA template containing a 5' Cy3 tag (green ball). The resulting nucleosome displays maximal FRET upon assembly. **(B)** The DNA template contains a 5' terminal nucleosome positioning sequence (grey) with a single GATC motif (green). TGGA repeats (orange) are used to support assembly on 5' DNA.

At its assembled starting position, the Cy3 and Cy5 fluorophores fall within the distance limit that generates maximum FRET signal (**Figure 5A**). Nucleosome remodeling separates the two fluorophores, leading to increased Cy3 signal and a concomitant loss of Cy5 signal. The ratio of Cy3 to Cy5 signal is used to measure nucleosome remodeling (see Section 4.4).

The DNA template also includes one GATC motif embedded within the NPS (**Figure 5B**), making it inaccessible to GATC-recognizing proteins in EpiDyne-FRET nucleosomes prior to remodeling. This feature enables validation of nucleosome positioning by restriction enzyme digestion (e.g. DpnII) if needed.

Applications: EpiDyne-FRET nucleosomes are specifically designed for compatibility with high-throughput FRET assays (Section 4.4), which provide robust and rapid results for compound screening. Because EpiDyne-FRET nucleosomes are terminally positioned, they are ideal for ATPases that redistribute terminal nucleosomes to central locations on DNA fragments (e.g. CHD remodelers, select ISWI remodeling complexes [ACF, CHRAC])[31,32,36-38] or enzymes with no preference (e.g. SMARCA2. SMARCA4[28]).

EpiDyne-FRET nucleosomes are also well-suited for the study of remodelers that exchange H2A histone variants (e.g. yeast SWR1[39]). In these experiments, H2A exchange can be measured by loss of FRET activity.

3.2 EpiDyne-GATC Terminal Nucleosome Remodeling Substrates

Background: The next category of remodeling substrate is EpiDyne-GATC Terminal Nucleosome Substrates, referring to their 5' position on DNA templates. EpiDyne-GATC Terminal nucleosomes are composed of an unmodified human histone octamer wrapped with a biotinylated or nonbiotinylated DNA template (see **Table 3**). These highly pure nucleosomes contain one, two, or three GATC motifs embedded in the 5' Widom 601 nucleosome positioning

sequence[34] (**Figure 6A and 6B**) to enable the use of GATC-recognizing enzymes (e.g. DpnII) for assay readout.

To reinforce terminal assembly, the 5' nucleosome positioning sequence is flanked by TGGA repeats, which are refractory to nucleosome wrapping[34,35]. An example of this 6-**N**-66 DNA template containing a single GATC motif is provided in **Figure 6B**, where **N** refers to **N**ucleosome positioning sequence; there are 6 bp upstream and 66 bp downstream of the final positioned nucleosome (6-**N**-66). Note that the 3' terminus also contains an acceptor sequence to accommodate the histone octamer after remodeling (not shown).

At its assembled starting position, GATC site(s) are protected by the histone octamer and inaccessible to GATC recognizing enzymes (see **Figure 6A**). As the histone octamer is relocated or ejected, the GATC site(s) are exposed and become functional substrates for secondary reactions, such as Dam DNA methylation and/or restriction enzyme digestion (DpnI, DpnII, MboI). Quantification of methylation or digestion can serve as readout for nucleosome remodeling.

Why would you need multiple GATC motifs? In methylation-coupled radiometric or direct DNA methylation assays, incorporation of multiple GATC sites increases the number of target epitopes per nucleosome and is expected to improve signal-to-background. EpiCypher has built various remodeling assays using GATC-Terminal nucleosomes to meet customer needs.

Applications: The EpiDyne-GATC Terminal nucleosomes are best suited for the study of ATPases that move nucleosomes from distal to central positions on DNA templates (e.g. CHD remodelers, select ISWI remodeling complexes [ACF, CHRAC])[31,32,36-38]. The SWI/SNF enzymes can also be studied using these substrates, since they lack terminal vs. central substrate specificity[28].

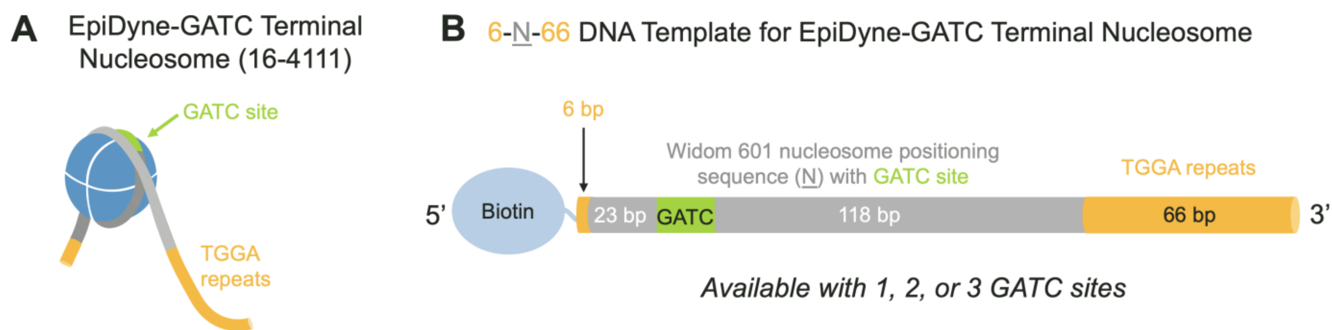


Figure 6. (A) EpiDyne-GATC Terminal nucleosome with one GATC motif (EpiCypher 16-4111). **(B)** The DNA template is biotinylated (light blue) and includes a single GATC motif (green) embedded in the 5' terminal nucleosome positioning sequence (grey). The 66 bp downstream of the nucleosome positioning sequence are refractory to nucleosome assembly (orange), reinforcing 5' nucleosome localization.

3.3 EpiDyne-GATC Central Nucleosome Remodeling Substrates

Background: The final set of remodeling substrates is the EpiDyne-GATC Central Nucleosome Substrates, indicating their central position on a biotinylated DNA template. These highly pure nucleosomes contain one, two, or three GATC motifs embedded in the central NPS (see **Table 3**, **Figure 7A and 7B)**, making them a useful tool for assays that apply GATC-recognizing enzymes to measure remodeling activity.

Similar to the EpiDyne-GATC Terminal nucleosomes (Section 3.2), these central nucleosomes use TGGA repeats to enforce central nucleosome assembly[34,35]. Specifically, the DNA template contains a central Widom 601 nucleosome positioning sequence[34] flanked by TGGA repeats. An example of this 50-**N**-66 template containing a single GATC motif is provided in **Figure 7B**, where **N** refers to **N**ucleosome positioning sequence; there are 50 bp upstream and 66 bp downstream of the correctly assembled nucleosome.

At the GATC-Central nucleosome starting position, the GATC site(s) are wrapped around the histone octamer and protected from GATC-recognizing enzymes (**Figure 7A**). As the histone octamers are relocated or ejected during nucleosome remodeling reactions, the GATC site(s) are exposed and become functional substrates for secondary enzymatic reactions like Dam DNA methylation and/or restriction enzyme digestion (DpnI, DpnII, MboI). Assays that monitor methylation or restriction digestion reactions can serve as a proxy for chromatin remodeling.

Applications: The EpiDyne-GATC Central nucleosomes are best suited for the study of ATPases that preferentially move nucleosomes from central to distal positions on DNA templates (e.g. the ISWI remodeling complex NURF[40]). The SWI/SNF enzymes SMARCA2 and SMARCA4 can also be studied using these nucleosomes, since both lack terminal vs. central substrate specificity[28].

(See Section 3.2 for information about the advantages of using multiple GATC sites)

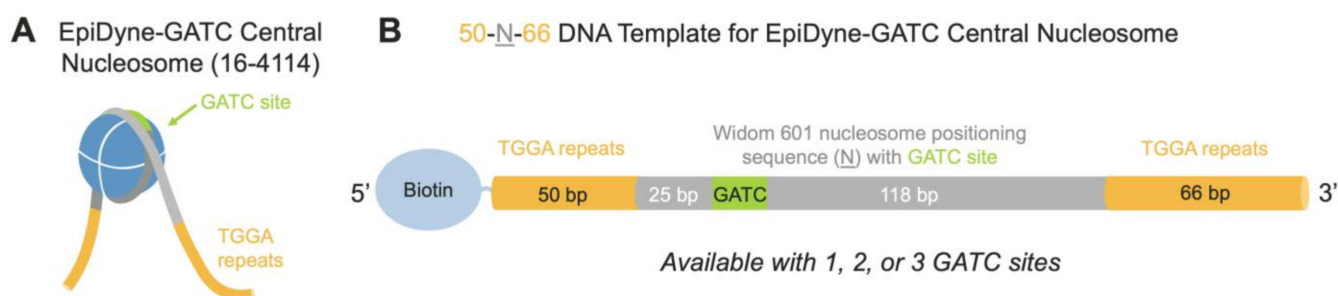


Figure 7. (A) EpiDyne-GATC Central nucleosome with one GATC motif (EpiCypher 16-4114). **(B)** The DNA template is biotinylated (light blue) and includes a single GATC motif (green) embedded in the 5' central nucleosome positioning sequence (grey). The 50 bp upstream and 66 bp downstream of the NPS are refractory to nucleosome assembly (orange), reinforcing central nucleosome localization.

4. EpiDyne Chromatin Remodeling Assays and Protocols

4.1 Substrate and Assay Selection

Before selecting and planning an EpiDyne remodeling experiment, consider what is already known about your ATPase. This includes information about nucleosome substrate preference and the type of mechanism you are going to study. If you aren't sure, see the **FAQs** below.

These factors will help narrow down suitable EpiDyne substrates and compatible EpiDyne assays (**Figure 8**). Always confirm that your nucleosome substrate will work in the desired endpoint assay. For instance, if your ATPase prefers centrally positioned nucleosomes, you will purchase EpiDyne GATC-Central substrates. These nucleosomes are only compatible with EpiDyne-PicoGreen and -REA assay formats; FRET assays are not an option (**Figure 8**).

Table 4 (next page) further summarizes the features of EpiDyne-REA, -FRET, and -PicoGreen chromatin remodeling assays detailed in this section.

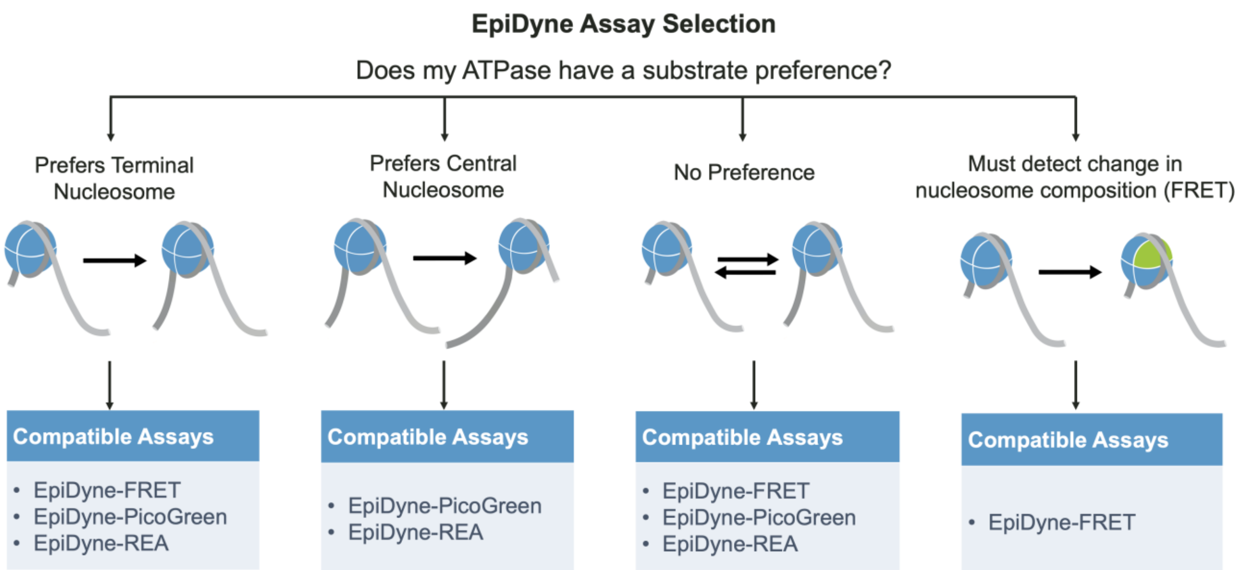


Figure 8. When selecting an EpiDyne assay, it is important to consider the ATPase substrate preference and other assay requirements. See additional information in **Table 4**, below.

Assay and substrate selection FAQs:

What if I don't know my enzyme's substrate preference? AND *What should I do if I am working with a novel or understudied enzyme?*
Many ATPases have not been fully characterized for nucleosome positioning preference. In these cases, or in instances where enzymes have failed to show activity in other remodeling assays, it is recommended to use EpiDyne-PicoGreen assays. EpiDyne-PicoGreen is

compatible with both terminal and central positioned nucleosomes (**Figure 8**), requires less enzyme (vs. REA), and is designed for higher throughput, making it ideal for initial studies. It is particularly important to define substrate preference when studying ISWI family remodelers, which often display strong terminal or central nucleosome preference (see Section 4.5).

I'm still confused. Are there other resources? EpiCypher is expert in chromatin remodeling assays – everything in this User Guide is from our capable scientists. For help with assay development and optimization, inquire about EpiDyne Assay Services at epicypher.com/EpiDyne-services.

4.2 EpiDyne Assay Limitations

Each assay approach has advantages and weaknesses to consider. Users must weigh the benefits and shortcomings of each approach and make the best decision for their project.

	REA	FRET	PicoGreen
Suitable Nucleosome	EpiDyne-GATC (Terminal & Central)	EpiDyne-FRET (Terminal Only)	EpiDyne-GATC (Terminal & Central)
Tested Activity	Nucleosome remodeling	Nucleosome remodeling	Nucleosome remodeling
Assay Readout	End point gel based imaging	Real time fluorescence	End point fluorescence
2˚ Enzyme	Restriction enzyme	None	Restriction enzyme
Operation Time	3-4 hours (1 hour hands-on)	~1 hour (30 min hands-on)	4-5 hours (2 hours hands-on)
S/B Range (ATP+ / ATP-)	ND	~1.5 - 3	~10 - 50
[Enzyme] per reaction	High	High	Low
Z' with SMARCA5	ND	~0.7	~0.8
Throughput	Low	High	High

Table 4. Overview of EpiDyne Assays, features, and applications. S/B, Signal over Background. ND, Not Determined.

- **EpiDyne-REA**: Restriction Enzyme Accessibility (REA) is an endpoint assay that depends on low throughput gel electrophoresis techniques and a secondary enzymatic digestion to detect chromatin remodeling. As an endpoint assay, only one measurement is recorded per reaction, meaning that time course experiments require multiple reactions – greatly increasing experimental costs. REA also uses a relatively high amount of enzyme and substrate, especially compared to EpiDyne-PicoGreen, which further adds to costs. Taken together, REA assays are incompatible with compound screening and HTS studies.

- **EpiDyne-FRET**: FRET assays suffer from autofluorescence, spectral overlap, and instrument background, which can narrow the detection window and result in false positives during compound screens. The no-wash, one-step assay format also increases risk of off-target compound interference, necessitating further counter-screening to confirm compound activity and specificity. Finally, because the EpiDyne-FRET substrate is terminally positioned, it is not suitable for remodeling ATPases that prefer centrally localized nucleosomes (e.g. ISWI/NURF complexes).

  Notably, the amount of enzyme used in EpiDyne-FRET is similar to lower-throughput EpiDyne-REA assays, which is suboptimal for HTS. However, since FRET assays record multiple timepoints per reaction (i.e. kinetic assay), the total amount of enzyme may be lower vs. REA experiments.

- **EpiDyne-PicoGreen**: EpiDyne-PicoGreen assays require multi-step operations, assay-specific equipment, and longer hands-on time (compared to other methods, above). This assay is also an endpoint detection method, meaning that more reactions are needed for time course remodeling experiments. The more complex protocol, combined with the need for more reactions, makes it less applicable for automated and/or parallelized workflows. However, given the strong advantages of EpiDyne-PicoGreen – including high signal-to-background, high sensitivity, and reduced enzyme consumption (**Table 4**) – these drawbacks may be acceptable trade-offs.

4.3 Developing and Optimizing EpiDyne Assays

Key optimization steps

- **Enzyme concentration**: It is recommended to titrate your remodeling enzyme to determine the ideal concentration for your experiment and assay format. EpiCypher selects enzyme concentrations that give linear results with increasing substrate concentration and generate the desired signal over background (see S/B in **Table 4**). For a positive control, use an EpiCypher remodeling enzyme and compatible substrate at recommended concentrations.
- **Substrate optimization**: Before performing an experiment, it is imperative to know if your enzyme shows substrate preference (see **Figure 8**). Incorrect substrates may result in poor signal and will not accurately reflect enzyme activity *in vivo*.
- **Substrate concentration**: It is recommended to perform substrate titrations, since some enzymes or assay formats require less substrate. EpiCypher selects the substrate concentration that gives reliable signal over background in the desired assay (see **Table 4**).

Developing HTS compatible assays for compound screening and drug development

Additional steps are required to confirm reliable and robust performance. **Figure 9** outlines the recommended steps for optimizing EpiDyne-FRET or EpiDyne-PicoGreen assays for HTS. If possible, include a validated EpiCypher enzyme and substrate as a positive control.

- **Substrate and enzyme conditions:** Substrate and enzyme conditions should be optimized as outlined above to determine ideal concentrations for linear kinetics[41]. This will help ensure accuracy and reproducibility of inhibitor data.
- **DMSO tolerance testing:** Most compound libraries or tool inhibitors are provided in DMSO, and HTS screens typically require compatibility with 1% DMSO (or more).
- **Assay reliability and reproducibility (Z' testing):** For HTS, assays should be developed for 96-, 384-, or 1536-well plates, and have low intra- and inter-assay variability. Z' testing can be used to determine suitability for HTS studies; Z' >0.6 is acceptable for HTS[42-45].
- **IC50 Testing with tool inhibitors:** For further functional validation, perform dose response curves with tool inhibitor compounds (if available) to calculate IC_{50} values[45].

EpiDyne HTS Assay Development and Optimization

Figure 9. Key steps for HTS assay optimization.

4.4 EpiDyne-FRET Assays

Background

EpiDyne-FRET was specifically developed to bridge the gap between basic chromatin remodeling research and drug development. The strategy uses Fluorescence Resonance Energy Transfer (FRET) as a readout, which provides high sensitivity, exquisite reproducibility, and fulfills the no-wash, homogeneous assay requirements for HTS. The assays are also continuous, allowing time-resolved studies of chromatin remodeling. Our highly pure EpiDyne-FRET nucleosomes (EpiCypher 16-4201; see Section 3.1) are uniquely designed for this strategy, offering a rigorously optimized physiological substrate. The combination of nucleosome substrates with HTS assays enables accurate, continuous detection of chromatin remodeling activity and will advance this exciting area of pharmaceutical research.

Assay Description

EpiDyne-FRET Nucleosomes (Section 3.1) are composed of a Cy5-tagged histone octamer wrapped with a DNA template containing a 5' Cy3 fluor. At the starting position, the octamer is located at the 5' terminal end of the DNA template (**Figure 10**). The proximity of the two fluorophores allows efficient FRET from Cy3 to Cy5, generating maximal Cy5 signal.

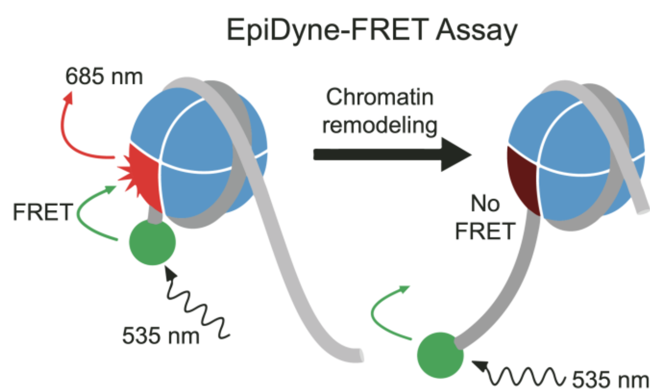


Figure 10. Schematic of EpiDyne-FRET nucleosome remodeling assay using EpiDyne-FRET nucleosomes substrate (EpiCypher 16-4201).

Addition of remodeling enzyme and ATP moves the Cy5 octamer away from the Cy3 DNA fluor, reducing FRET efficiency (i.e. increase of Cy3 signal and a reciprocal decrease of Cy5 signal). The assay is monitored using Cy3 excitation and simultaneous detection of Cy3 and Cy5 emission signals on a 384-well plate reader. Data are expressed as the ratio Cy3/Cy5 signal; successful remodeling is represented by increasing Cy3/Cy5 signal over time (**Figure 11**).

Readout and Example Data

EpiDyne-FRET uses the ratio of Cy3/Cy5 emissions to define time, ATP, and enzyme dose-dependent increases in chromatin remodeling (**Figure 11**). EpiCypher remodeling enzymes are well-suited for EpiDyne-FRET assays and have been used to demonstrate the high reproducibility and consistency of this platform for HTS workflows (**Figure 12**). EpiDyne-FRET is also compatible with drug screening, as represented by a dose response curve of the published SMARCA2/4 specific inhibitor BRM-014 (**Figure 12**)[1,2].

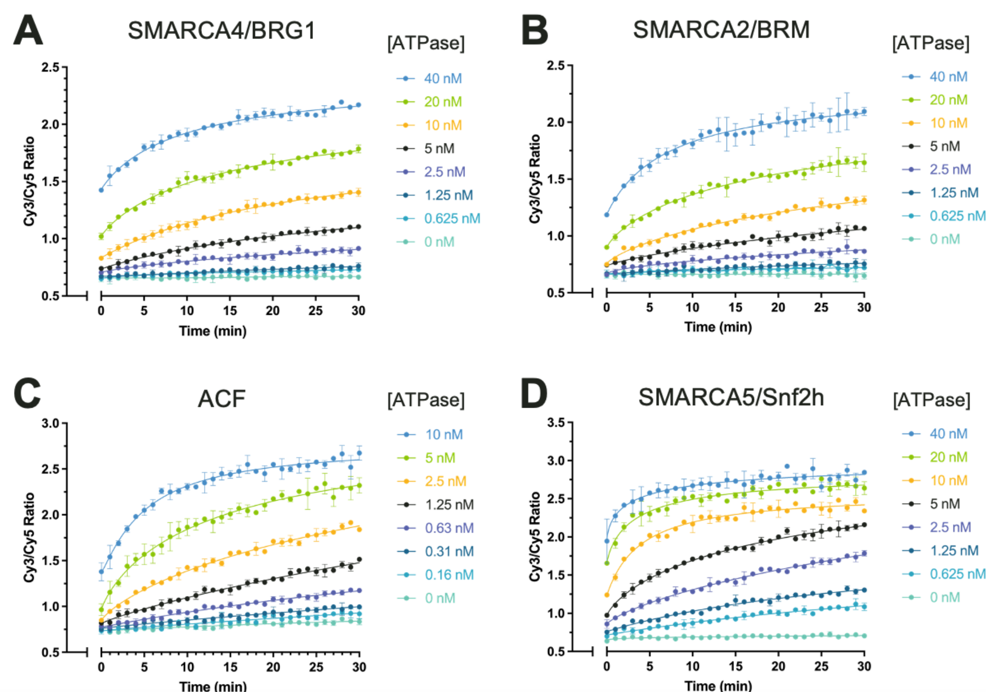
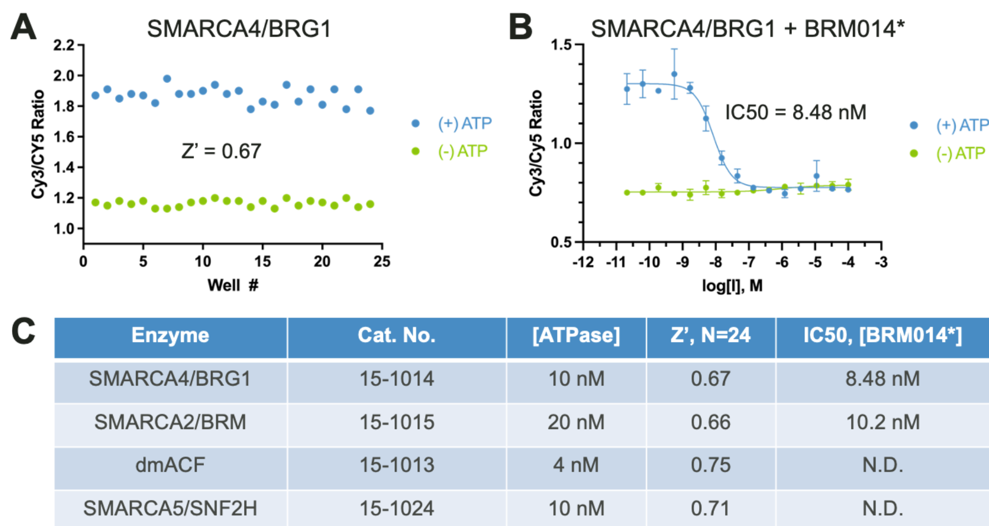


Figure 11. EpiDyne-FRET assays are used to study time- and enzyme dose-dependent chromatin remodeling reactions by **(A)** SMARCA4/BRG1 (EpiCypher 15-1014), **(B)** SMARCA2/BRM (15-1015), **(C)** ACF complex (15-1013) and **(D)** SMARCA5/SNF2H (15-1024).



Enzyme	Cat. No.	[ATPase]	Z', N=24	IC50, [BRM014*]
SMARCA4/BRG1	15-1014	10 nM	0.67	8.48 nM
SMARCA2/BRM	15-1015	20 nM	0.66	10.2 nM
dmACF	15-1013	4 nM	0.75	N.D.
SMARCA5/SNF2H	15-1024	10 nM	0.71	N.D.

*BRG1/BRM specific compound BRM014 [Novartis]: Papillon et al. 2018

Figure 12. Application of EpiDyne-FRET for high-throughput screening. **(A)** EpiDyne-FRET assays for SMARCA4/BRG1 have Z' values >0.6, indicating strong reproducibility for HTS. **(C)** Analysis of other EpiCypher ATPases using EpiDyne-FRET shows similar Z' values. **(B)** EpiDyne-FRET recapitulates strong inhibition of SMARCA4 by the compound BRM-014 (IC50=8.48 nM). **(C)** IC50 testing confirmed the specificity of BRM-014 for SMARCA4 and SMARCA2[1,2]. ND, not determined.

EpiDyne-FRET Protocol using EpiDyne-FRET Nucleosomes and SMARCA4/BRG1

The protocol below was developed by EpiCypher scientists using EpiDyne-FRET nucleosome substrates and SMARCA4/BRG1 enzyme. We suggest validating this assay in your lab during initial experiments. Further optimization may be required for other enzymes; see below.

Assay Setup and Optimization Notes

1. For assay development it is recommended to titrate concentrations of remodeling enzyme and substrate. This will help determine optimal conditions and establish a linear range.
2. It is recommended to use an EpiCypher remodeling enzyme as a positive control.
3. Include a "No-ATP" negative control whenever possible.
4. It is advised to use 1 mM ATP for EpiCypher remodeling enzymes (i.e. at saturation) to ensure that nucleosome substrates are the limiting factor for assay signal. For other applications or non-EpiCypher enzymes, titrate ATP to determine optimal working concentrations.
5. ATPγS, a nonhydrolyzable form of ATP, can be used as a negative control. Note that this control should not be used to directly assess or compare nucleosome remodeling capabilities.
6. Reactions can begin rapidly and typically complete within 30 minutes. Therefore, it is important to start reading as soon as the reaction is initiated. If setting up a large plate without an auto-injector, it will not be possible to collect a true time zero (T0) measurement for each reaction.
7. Fluorescent detection is highly subject to equipment and filter setting variations. It is critical to note that only the change in the Cy3/Cy5 ratio (by time and/or following addition of ATP) should be used to determine assay sensitivity or performance. Steady-state signals are not indicative of assay success.

Required Reagents & Materials

Note: Store protein aliquots at -80°C and avoid multiple freeze-thaw cycles.

- SMARCA4/BRG1 remodeling enzyme (EpiCypher 15-1014; epicypher.com/15-1014)
  - Protocol is also compatible with human SMARCA2/BRM and SMARCA5/SNF2H
- EpiDyne-FRET nucleosome substrate (EpiCypher 16-4201, epicypher.com/16-4201); see TDS for molarity of nucleosome (varies lot-to-lot)
- ATP, Tris-buffered (Thermo Fisher R1441)
- 1X Remodeling Buffer (20 mM Tris pH 7.5, 50 mM KCl, 3 mM $MgCl_2$, 0.01% Tween-20, 0.01% BSA); a 5X stock of this buffer is provided with the SMARCA4 enzyme
- Black 384-well assay microplate (Corning 3820; make sure low-volume)
- Multi-channel pipettors and tips
- Cy3 (Excitation 531/Emission 579) and Cy5 (Emission 685) compatible plate reader
  - EpiCypher uses the Envision 2104 plate reader. Filter wavelength/bandpass (nm) parameters: Excitation (531/25), first emission (579/25); second emission (685/35).

Basic Protocol (~1 hour with 30 min hands-on time)

1. Prepare 1X Remodeling Buffer and equilibrate to room temperature.
2. Prepare stocks of assay components in 1X Remodeling Buffer:
    - 4X SMARCA4/BRG1: Use 4X concentration on TDS suggested for FRET (varies lot-to-lot). Need 2.5 µL per reaction.
    - 4X EpiDyne-FRET nucleosomes: 80 nM, need 2.5 µL per reaction; titrate if needed.
    - 2X ATP: 2 mM, need 5 µL per reaction.
3. Assign reactions to wells of a black 384-well microplate. Add 2.5 µL 4X SMARCA4/BRG1 and 2.5 µL 4X EpiDyne-FRET Nucleosome per reaction.
4. Set up plate reader to excite at 531 nm and read Cy3 (579 nm) and Cy5 (685 nm) emissions. The first plate read should be at 0 seconds (T0). For real time monitoring, set up the plate reader to read once every 60 seconds for 30 minutes.
5. Add 5 µL 2X ATP to each reaction to initiate remodeling. For No-ATP control reactions, add 5 µL 1X Remodeling Buffer.
6. Spin down plate (to collect droplets) and IMMEDIATELY start reading on the plate reader.
7. Express data as the ratio of the raw Cy3/Cy5 emission signals at each timepoint.

Troubleshooting Guide

Observation	Possible Cause	Troubleshooting Suggestions
Cy3/Cy5 signal is noisy, high background	• Signal counts are too low resulting in fluctuation • Background interference from 384-well plates	• Adjust plate reader protocol to detect >10,000 raw counts in both Cy3 and Cy5 channels to minimize fluctuations • Ensure black, low-volume 384-well plates are used • Avoid re-using old plates; replace plates if necessary
No change in Cy3/Cy5 over time progression or with enzyme titration	• Nucleosome disassembly • Remodeling enzyme inactive	• Use fresh nucleosome aliquots. Avoid repeated freeze/thaw • Move quickly to minimize nucleosome time in buffer • Include a positive control enzyme (e.g. EpiCypher 15-1013, 15-1024) • Replace remodeling enzyme if possible
Cy3/Cy5 signal is enzyme-dose dependent, but does not change over time	• Detection is not rapid enough to capture early kinetics of enzymatic reaction	• Read plate immediately after ATP addition. Take continuous reads, no more than 2 min apart • Reduce ATP to slow the remodeling reaction • If kinetic reading is not feasible, compare end-point readouts between + and - ATP reactions

4.5 EpiDyne-PicoGreen™ Assay

Background

The EpiDyne-PicoGreen™ Assay (under provisional patent protection by EpiCypher) is a versatile chromatin remodeling assay that combines restriction enzyme accessibility and biotinylated nucleosome substrates with a high-throughput quantitative readout. Although EpiDyne-PicoGreen is an endpoint assay, it has unprecedented advantages in sensitivity and consumes significantly less enzyme compared to EpiDyne-FRET and -REA. The assay also involves stringent washes between remodeling reactions and final detection, reducing compound interference and assay background to enable HTS compatible workflows[46].

Assay Description

The EpiDyne-PicoGreen assay is compatible with EpiDyne-GATC Terminal (Section 3.2) and Central (Section 3.3) nucleosomes, which are wrapped with a biotinylated DNA template that carries one to three GATC restriction enzyme motifs in the nucleosome positioning sequence. At the assembled starting position GATC sites are protected from enzyme digestion. Nucleosome remodeling alters the position of the histone octamer on the DNA template, exposing the GATC sites.

After remodeling, the biotinylated nucleosomes are immobilized to streptavidin-coated magnetic beads and washed repeatedly to minimize assay background. Bead-bound nucleosomes are incubated with a GATC-recognizing enzyme (e.g. DpnII), which cleaves DNA on remodeled nucleosomes. The portion of biotinylated DNA remains bound to the magnetic bead, while the cleaved nucleosome is released into the supernatant (**Figure 13**).

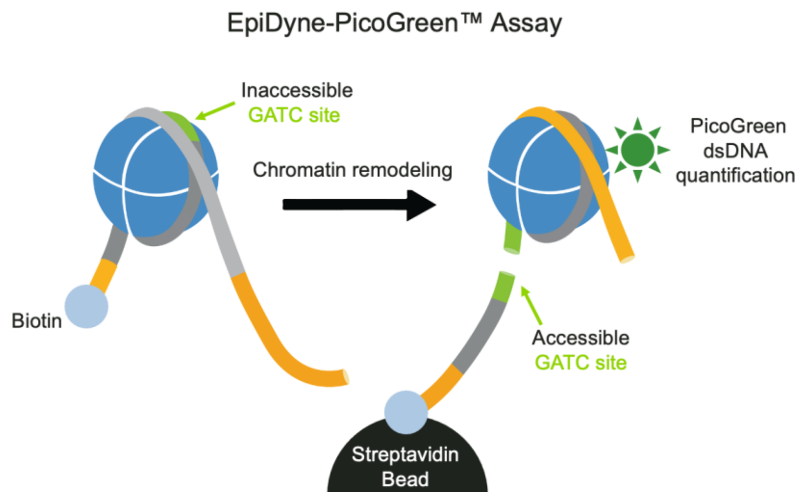


Figure 13. Schematic of the EpiDyne-PicoGreen nucleosome remodeling assay, using the EpiDyne-GATC Terminal nucleosome with one GATC site as a substrate (EpiCypher 16-4111).

The supernatant is separated from beads and used for PicoGreen fluorescence assays, which apply the PicoGreen nucleic acid intercalator to quantify double-stranded DNA with ultra-high sensitivity. Successful remodeling correlates with elevated PicoGreen fluorescent signal in the assay supernatant (**Figure 14**).

Readout and Example Data

EpiDyne-PicoGreen reports active nucleosome remodeling as function of time, ATP, and enzyme dose-dependent increases in PicoGreen fluorescence, with a typical signal-to-background ratio above 5 (**Figure 14**). Due to its high sensitivity, the EpiDyne-PicoGreen assay format is uniquely capable of comparing nucleosome substrate preferences across different remodeler enzyme families. Linear regression slopes can be plotted to illustrate initial rates of remodeling for enzyme comparisons. For instance, **Figure 15A and B** show SMARCA2 does not distinguish between EpiDyne-GATC Terminal and Central nucleosomes, while SMARCA5 (an ISWI ATPase) clearly favors EpiDyne-GATC Terminal nucleosomes. In contrast, *Drosophila* ISWI (NURF complex) prefers GATC Central nucleosomes (**Figure 15C**; in agreement with prior studies[31,47]). Taken together, these results underscore the differences between remodelers both within and across enzyme families.

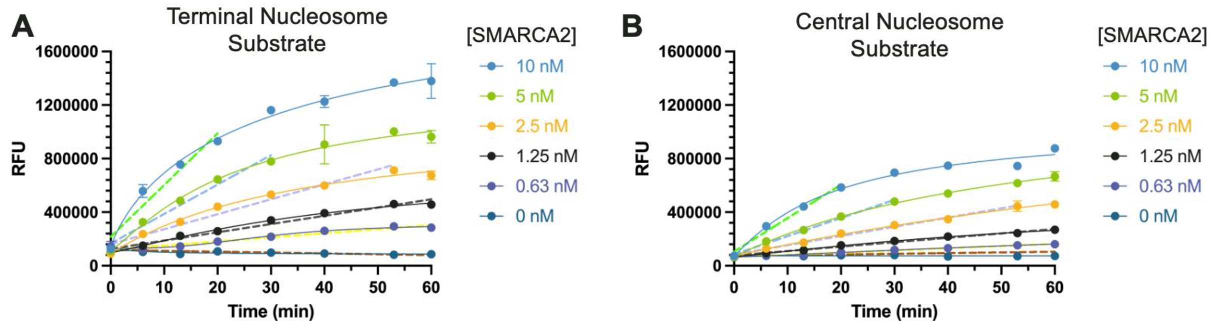


Figure 14. EpiDyne-PicoGreen assays show time- and dose-dependent remodeling of EpiDyne-GATC Terminal **(A)** and Central **(B)** nucleosomes by SMARCA2/BRM (EpiCypher 15-1015).

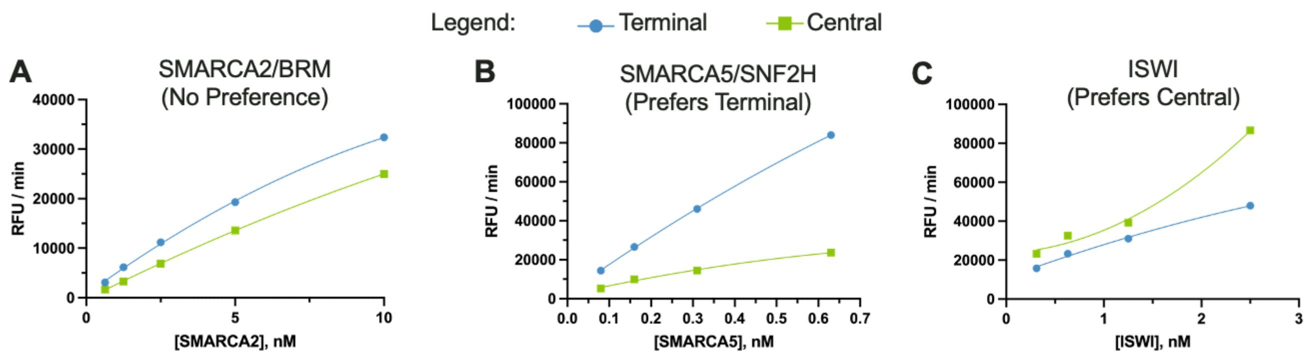
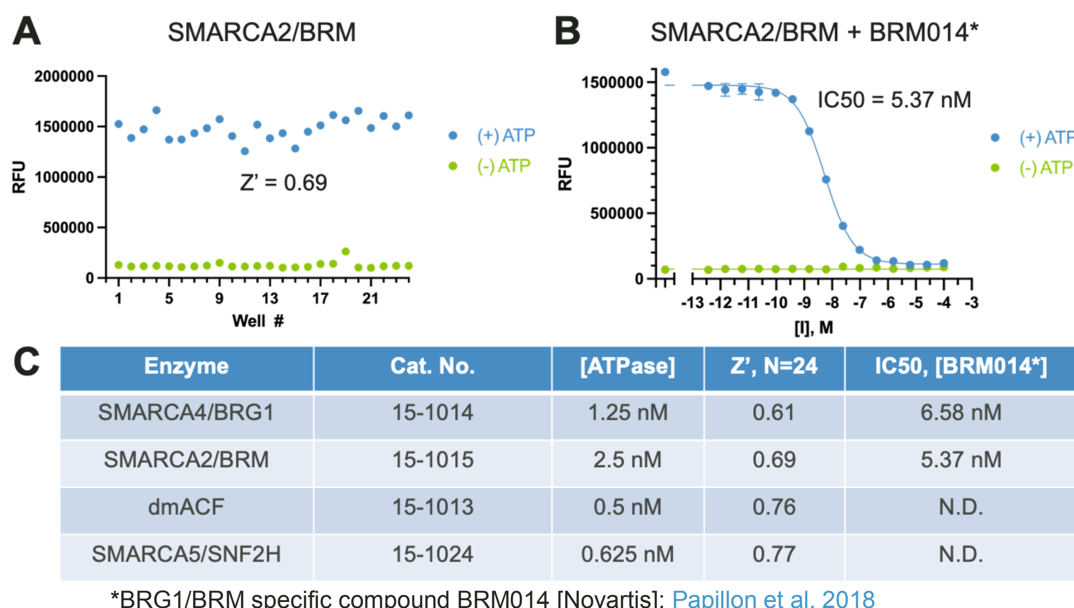


Figure 15. Initial remodeling rates from EpiDyne-PicoGreen assays reveal substrate preferences of chromatin remodeling enzymes. **(A-C)** Graphs show changes in initial remodeling rates as a function of increasing enzyme concentration. Reaction rates (RFU/min) were generated for individual enzyme concentrations using the slope of time-dependent chromatin remodeling (e.g. for SMARCA2, data taken from enzyme titrations in **Figure 14**). **(A)** SMARCA2/BRM shows no preference for EpiDyne-GATC Terminal vs. Central nucleosomes, as expected. **(B,C)** Although both SMARCA5/SNF2H and ISWI are ISWI family ATPases, these two enzymes show divergent substrate preferences.

EpiDyne-PicoGreen has high reproducibility and consistency, making this platform suitable for HTS applications. To demonstrate these capabilities, we determined the Z' score of EpiDyne-PicoGreen using EpiCypher's collection of remodeling enzymes (**Figure 16A, C**). Each assay had a Z'> 0.6, supporting use for high-throughput projects. To further establish compatibility with drug and compound screening, we used EpiDyne-PicoGreen to perform dose-response curves of a published specific SMARCA2/4 inhibitor BRM-014 (Novartis)[1] with each ATPase. As shown in IC50 data provided in **Figure 16B and 16C**, the compound had strong inhibitory effects on SMARCA4 and SMARCA2, but not the ISWI remodelers SMARCA5 or ACF[1,2].



Enzyme	Cat. No.	[ATPase]	Z', N=24	IC50, [BRM014*]
SMARCA4/BRG1	15-1014	1.25 nM	0.61	6.58 nM
SMARCA2/BRM	15-1015	2.5 nM	0.69	5.37 nM
dmACF	15-1013	0.5 nM	0.76	N.D.
SMARCA5/SNF2H	15-1024	0.625 nM	0.77	N.D.

*BRG1/BRM specific compound BRM014 [Novartis]: Papillon et al. 2018

Figure 16. EpiDyne-PicoGreen assays are suitable for HTS and compound screening. **(A,C)** Z' reproducibility testing shows scores >0.6 for all EpiCypher ATPase remodeling enzymes. **(B,C)** EpiDyne-PicoGreen assays and IC50 testing confirms that BRM-014 is a specific inhibitor of SMARCA2 and SMARCA4 (low nM IC50 values). ND, not determined.

EpiDyne-PicoGreen Protocol using EpiDyne-GATC Nucleosomes and Select Enzymes

This protocol was developed at EpiCypher. We suggest validating this protocol in your lab during initial experiments. Further optimization may be required for non-EpiCypher enzymes.

Assay Setup and Optimization Notes

1. EpiDyne-PicoGreen assays support end-point readouts. For time progression studies, set up reactions for each timepoint. Add Quench Buffer and beads at the assigned times.
2. For "Time = 0" samples, add Quench Buffer and beads to reaction before adding ATP.
3. Include a "No ATP" negative control whenever possible.
4. Note that nucleosome substrates with one, two, and three GATC sites will generate similar results in EpiDyne-PicoGreen assays; additional GATC motifs do NOT increase final signal.
5. For assay development it is recommended to titrate the concentration of remodeling enzyme and substrate. This will determine optimal conditions and establish a linear range.

6. It is recommended to use an EpiCypher remodeling enzyme as a positive control.
7. It is recommended to use 1 mM ATP for EpiCypher remodeling enzymes (i.e. at saturation), to ensure that the nucleosome substrate is the limiting factor for assay signal. For other applications or non-EpiCypher enzymes, titrate ATP to determine optimal concentration.
8. ATPγS, a nonhydrolyzable form of ATP, can be used as an inhibitor in EpiDyne-PicoGreen assays. Note that this control does not directly reflect the nucleosome remodeling reaction.
9. Ensure Streptavidin beads are evenly mixed for long incubations:
   - Shake 384-well plate at 1,000 RPM for <2 min and check that beads are resuspended.
   - Incubate 384-well plate on a nutator. Do not exceed 3 hours of mixing on a nutator (nucleosomes on beads become unstable). If necessary, use multiple 384-well plates.

Required Reagents & Materials

Note: Store protein aliquots at -80°C and avoid multiple freeze-thaw cycles.

- SMARCA2/BRM remodeling enzyme (EpiCypher 15-1015)
  - Assay is also compatible with human SMARCA4/BRG1 and SMARCA5/SNF2H
- EpiDyne-GATC Terminal nucleosomes (e.g. EpiCypher 16-4111 and 16-4114); see TDS for molarity (varies lot-to-lot)
  - Assay is compatible with all biotinylated EpiDyne-GATC nucleosome substrates
- ATP, Tris-buffered (Thermo Fisher R1441)
- 1X Remodeling Buffer (20 mM Tris pH 7.5, 50 mM KCl, 3 mM $MgCl_2$, 0.01% Tween-20, 0.01% BSA); a 5X stock of this buffer is provided with the SMARCA2 enzyme
- PicoGreen Quench Buffer (20 mM Tris pH 7.5, 600 mM KCl, 0.01% Tween-20)
- PicoGreen Wash Buffer (20 mM Tris pH 7.5, 300 mM KCl, 0.01% Tween-20)
- DpnII Buffer (20 mM Tris pH 7.5, 50 mM KCl, 3 mM $MgCl_2$, 0.01% Tween-20)
- Restriction enzyme DpnII (NEB R0543M; 50,000 Units/mL; will dilute 1:1,000 for digest)
- Clear 384-well assay microplate (Greiner Bio-One 781201)
- Streptavidin hydrophilic magnetic beads (4 mg/mL)
- 384-well plate compatible magnet (V&P)
- Quant-iT PicoGreen dsDNA Reagent
- Thermolabile Proteinase K
- TE buffer (10 mM Tris pH 7.5, 1 mM EDTA)
- Black 384-well detection microplate (Corning 3820)
- Nutator
- PicoGreen (Excitation 502 / Emission 523) compatible fluorescent plate reader

Basic Protocol (4-5 hours, with 2 hours of hands-on time)

1. Prepare buffers as outlined on the previous page and equilibrate to room temperature (RT).
2. Prepare assay components in 1X Remodeling Buffer:
   - 4X remodeling enzyme: PicoGreen typically requires ¼ enzyme used in FRET or REA (see lot-specific enzyme TDS). Need 5 µL per reaction.

- 4X GATC Terminal nucleosomes: 80 nM concentration, need 5 µL per reaction.
- 2X ATP: 2 mM concentration, need 10 µL per reaction.

3. Prepare Streptavidin hydrophilic beads: Mix 4 mg/mL bead stock and take 10 µL per reaction. Equilibrate beads in PicoGreen Quench Buffer as per manufacturer instructions to a final concentration of 2 mg/mL.
4. Assign reactions to wells of a clear 384-well plate. (Timepoints require separate reactions.)
5. Add 5 µL 4X Remodeling Enzyme and 5 µL 4X GATC Terminal nucleosome per reaction.
6. Add 10 µL 2X ATP per reaction to initiate remodeling. Note differences for controls:
   - No-ATP control: Add 10 µL 1X Remodeling Buffer.
   - T=0 control: Add 20 µL 2mg/mL Streptavidin beads (Step 3) before adding 10 µL ATP.
7. Incubate plate at RT. If using a T=0 control, place plate on a nutator to keep beads in solution.
8. At each timepoint, add 20 µL 2 mg/mL Streptavidin beads to designated reaction and incubate plate on a nutator. After final timepoint, incubate plate for one hour on a nutator.
9. Capture beads on 384-well plate magnet. Remove and discard supernatant.
10. Keeping the plate on the magnet, add 20 µL PicoGreen Wash Buffer per reaction. Remove and discard supernatant. Repeat two more times.
11. With plate on the magnet, add 20 µL DpnII Buffer to each reaction. Remove and discard supernatant and take plate off magnet.
12. Resuspend each reaction in 20 µL DpnII Buffer containing 50 Units/mL DpnII.
13. Incubate at RT for 30 min.
14. Return plate to magnet. Transfer 10 µL each supernatant to a black 384-well plate.
15. To each supernatant, add 10 µL TE buffer with 2X Quant-iT PicoGreen reagent and 2 Units/mL Thermolabile Proteinase K (final concentration of 1 Unit/mL).
16. Use plate reader to excite at 502 nm and read fluorescein emission (523 nm). Collect data expressed as relative fluorescence units (RFU).

Troubleshooting Guide

Observation	Possible Cause	Troubleshooting Suggestions
High signal fluctuation	• Reactions not evenly mixed at critical steps	• Ensure no bead clumps are seen after each resuspension: shake plates at 1,000 RPM until beads are evenly dispersed.
High background in control reactions	• Nucleosome disassembly • High plate background signal	• Use fresh nucleosome aliquots. Avoid repeated freeze/thaw. • Move quickly to minimize nucleosome time in buffer. • Use EpiCypher recommended assay plates.
Low signal in remodeling reactions	• Possible loss of sample during bead washes • Remodeling enzyme inactive	• Confirm beads are fully immobilized (i.e. solution is clear) and tightly secure assay plates on magnets before removing buffer/performing wash steps. • Avoid repeated freeze/thaw of enzymes. • Include positive control enzyme (EpiCypher 15-1013, 15-1024). • Replace enzyme if needed.

4.6 EpiDyne-REA

Background

Restriction Enzyme Accessibility (REA) assays use restriction digests to detect endpoint changes in nucleosome positioning on DNA templates. Nucleosome substrates for these assays contain restriction enzyme motifs in the nucleosome positioning sequence wrapped around the histone octamer, blocking recognition by enzymes. Remodeling exposes motifs for cleavage by the restriction enzyme, which can be monitored by gel electrophoresis. Increased restriction enzyme digestion thus serves as a direct readout for remodeling activity (**Figure 17**).

REA assays are one of the most widely used biochemical approaches to interrogate nucleosome remodeling. Although not suitable for high throughput screening, REA assays are straightforward, use readily available lab equipment (e.g. PAGE gel systems), and provide robust results.

Assay Description

EpiDyne-GATC Terminal and Central nucleosomes (Section 3.2 and Section 3.3) contain one, two or three GATC motifs within the nucleosome positioning sequence. At the starting state, DNA is refractory to digestion by DpnII, which cuts double-stranded DNA at unmethylated GATC sites. Upon successful octamer repositioning or ejection, GATC sites are exposed, providing a suitable substrate for DpnII digestion **(Figure 17**).

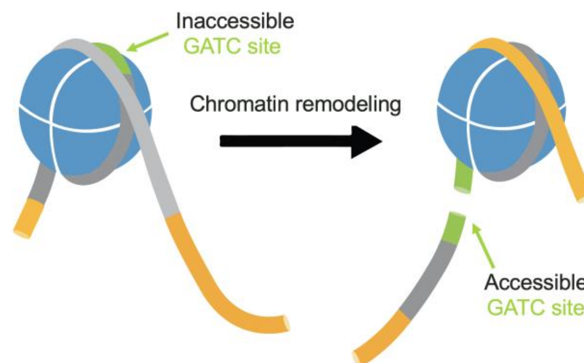


Figure 17. Schematic of EpiDyne-REA assay using EpiDyne-GATC terminal nucleosome substrates with one GATC site (EpiCypher 16-4111).

The EpiDyne-REA reaction is initiated by mixing remodeling enzyme, nucleosome substrates, ATP and DpnII in a single tube. At desired timepoints, the reaction is quenched by addition of EDTA, SDS and proteinase K. DNA from each timepoint is resolved on an 8% native PAGE gel. As shown in **Figure 18** (next pages), unremodeled nucleosomes have intact DNA while remodeled nucleosomes are cleaved by DpnII and show fragmented DNA. The ratio of fragmented to intact DNA at each time point reflects the rate of nucleosome remodeling.

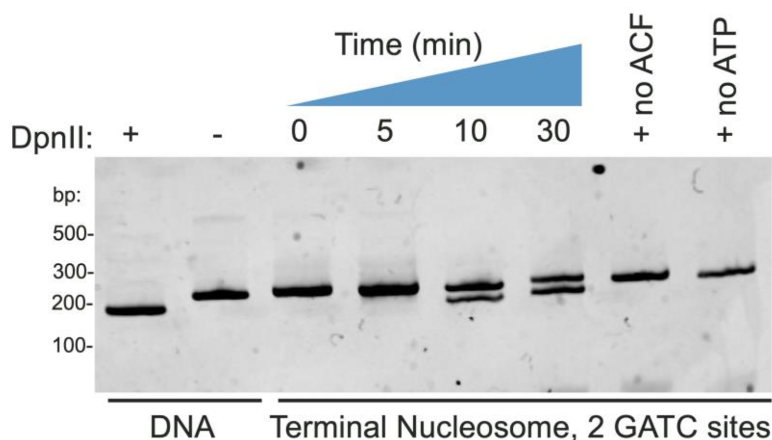
Readout and Example Data

Digested nucleosomal DNA is the readout for chromatin remodeling in EpiDyne-REA assays using *Drosophila* ACF (shown in **Figure 18**). Free DNA template is completely digested by restriction enzymes (Lane 1), while there is no cleavage from negative controls (Lanes 2, 7, 8). Successful remodeling is represented by a time dependent accumulation of the cleaved DNA species (Lanes 3-6). Nucleosomes with >1 GATC sites generate multiple cleavage products.

Figure 18. EpiDyne-REA assays demonstrate successful ATP-dependent nucleosome remodeling by ACF.

Lanes 1 - 2: Free DNA (EpiCypher 18-4112; 100 ng) with or without DpnII shows the migration range of the assay.

Lanes 3 - 8: EpiDyne-GATC Terminal nucleosomes with two GATC sites (16-4112) were incubated with 20 nM ACF for up to 30 minutes in the presence of 2 mM ATP and 50U DpnII. Samples were quenched at specified intervals and resolved via 8% native PAGE. No ACF and no ATP are negative controls.



EpiDyne-REA Protocol using EpiDyne-GATC Terminal Nucleosomes and *Drosophila* ACF

This protocol was developed at EpiCypher using our EpiDyne-GATC Nucleosome substrates and EpiDyne remodeling enzymes. We suggest validating this protocol in your lab during initial experiments and optimizing enzyme or substrate conditions as necessary.

Recommended Control Samples

- Use appropriate EpiDyne DNA templates as controls for restriction enzyme digestion. See Table 3 for recommendations.
- Run a “No-enzyme” negative control.
- Run a “No-ATP” negative control whenever possible.
- Include a “Pre-quenched” control with 20 µL Quench Buffer added before ATP.

Required Reagents & Materials

Note: Store protein aliquots at -80°C and avoid multiple freeze-thaw cycles.

- ACF remodeling complex (EpiCypher 15-1013)
  - Assay is also compatible with human SMARCA2 and SMARCA4
- EpiDyne-GATC Terminal nucleosome substrate (EpiCypher 16-4112); see TDS for molarity of nucleosome (varies lot-to-lot)
  - Assay is compatible with all EpiDyne-GATC nucleosome substrates
- Positive Control DNA Template (EpiCypher 18-4112)
- ATP, Tris-buffered (Thermo Fisher R1441)

- Restriction enzyme DpnII (NEB R0543M) or MboI (NEB R0147M)
- 1X Remodeling Buffer (20 mM Tris pH 7.5, 50 mM KCl, 3 mM $MgCl_2$, 0.01% Tween-20, 0.01% BSA); a 5X stock of this buffer is provided with the ACF remodeling enzyme
- 2X Quench Buffer (make fresh: 10 mM Tris pH 7.5, 40 mM EDTA, 0.6% SDS and 50 µg/mL Proteinase K)
- Heat block or thermocycler set to 55˚C
- 8% Native Polyacrylamide Gel (37.5:1) assembled/resolved in 1X TBE
- Ethidium Bromide (EtBr, 10 mg/mL) and appropriate imaging device

Basic Protocol (3-4 hours with 1 hour of hands-on time)

1. Prepare buffers as outlined above and equilibrate to room temperature (RT).
2. Prepare 4X stocks of assay components in 1X Remodeling Buffer:
   - 4X ACF: Use 4X concentration on TDS suggested for FRET (varies lot-to-lot). Need 5 µL per reaction. It is recommended to titrate enzyme in initial experiments.
   - 4X GATC Terminal nucleosomes: 800 nM, need 5 µL per reaction; titrate if needed.
   - 4X DpnII: 10 units/µL, need 4 µL per reaction.
   - 4X ATP: 4mM, need 5 µL per reaction.
3. In a clean 1.5 mL tube or 8-strip tube, mix 5 µL 4X ACF, 5 µL 4X GATC Terminal nucleosome, and 5 µL 4X DpnII.
4. To initiate remodeling reaction, add 5 µL 4X ATP. For No-ATP controls, add 5 µL 1X Remodeling Buffer.
5. At desired time points, add 20 µL 2X Quench Buffer to stop reaction.
6. Heat reactions for 20 min at 55˚C to remove proteins.
7. Resolve final product on a non-denaturing polyacrylamide gel (e.g. 105 volts for ~1 hour).
8. Stain gel with Ethidium Bromide (0.2 µg/mL) in 1X TBE for 10 min.
9. Destain the gel in 1X TBE and visualize on a compatible imaging device.

Troubleshooting Guide

Observation	Possible Cause	Troubleshooting Suggestions
DNA template control is not digested	• Restriction enzyme inactive • Gel system has low resolution • Control DNA was degraded	• Replace restriction enzyme or use a new aliquot • Use fresh gels, run at 105 volts for 1 hour • Replace aliquot of control DNA
No ATP control reaction is digested	• Nucleosome disassembly • DNase contamination in buffers	• Use fresh nucleosome aliquots that have not been repeatedly freeze/thawed • Prep Remodeling Buffer with DNase-free ingredients
No remodeling was observed	• Remodeling enzyme inactive	• Include positive control enzyme (e.g. EpiCypher 15-1013) • Replace remodeling enzyme, if possible • Prepare EDTA-free Remodeling Buffer to ensure adequate $MgCl_2$ concentrations

5. Tool Compounds in SWI/SNF Remodeler Research

Despite the overwhelming link between SWI/SNF chromatin remodelers and disease, the development of tool inhibitors targeting these complexes has stalled. This is largely due to the lack of sensitive, high-throughput, nucleosome-based chromatin remodeling assays. Recent literature has made significant progress on this front, with multiple inhibitors of the SWI/SNF ATPases SMARCA2 and SMARCA4. Below we detail these compounds and applications.

ATPγS

ATPγS is a stable analog of ATP that is very slowly hydrolyzed by ATPases and is used as a negative control in ATPase assays. As an ATP-competitive inhibitor, it does not show specificity for any remodeling enzymes and is best for general analysis of ATPase activity.

PFI-3

The chemical probe PFI-3 was initially described as a competitive inhibitor of the SMARCA2/4 bromodomain, blocking SMARCA2/4 binding to acetylated histone peptides[48]. However, in follow-up studies, PFI-3 failed to block full-length SMARCA2 binding and remodeling on recombinant nucleosomes, and did not replicate SMARCA2/4 deletion phenotypes. These results indicate that PFI-3 does not block SMARCA2/4 remodeling *in vivo* and has limited therapeutic potential[49].

BRM-014

The commercially available BRM-014 is an allosteric dual inhibitor of SMARCA2/4 ATPases developed by the Novartis Institute for Biomedical Research (NIBR). It has been validated for both biochemical and cellular studies and shows potent toxicity in SMARCA4-mutant lung cancer xenografts *in vivo*[1,2]. EpiCypher has validated the specificity of this inhibitor for SMARCA2/4 in EpiDyne-FRET and PicoGreen and observes consistent low-nM IC50 values.

ACBI1*

The commercially available ACBI1 is a Proteolysis Targeting Chimera (PROTAC) comprising a VHL ubiquitin ligase binding-ligand fused to a ligand that binds the SMARCA2/4 bromodomain. ACBI1 induces specific, targeted degradation of SMARCA2, SMARCA4 and the PBAF complex subunit PBRM1, and induced apoptosis across diverse cancer cell lines[50].

AU-15330*

AU-15330 is a commercially available PROTAC composed of the VHL ubiquitin ligase binding-ligand and a ligand that binds to the SMARCA2/4 bromodomain PBRM1[51], similar to ACBI1. The results suggest that degradation of SMARCA2/4 disrupts enhancer-driven oncogenic gene expression programs, leading to cancer cell death[51].

*These compounds are **NOT** directly compatible with EpiDyne assays but can be readily applied to cell-based assays.

6. Advanced Developments in Remodeler Research

Effectively targeting remodelers for drug development requires research that is complementary to the mechanistic data generated by EpiDyne assays. For instance, knowing the genome-wide localization of remodeling ATPases is crucial to understanding impacts on gene expression in healthy tissues vs. disease. However, current chromatin mapping approaches (i.e. ChIP-seq) are unreliable and difficult to optimize for remodelers, further impeding drug discovery.

EpiCypher's ultra-sensitive CUTANA™ CUT&RUN genomic mapping assays provide a robust approach to interrogate chromatin remodeling enzymes and is compatible with all four classes of remodeling ATPases (**Figure 19**). EpiCypher offers CUTANA™ CUT&RUN Antibodies to diverse chromatin remodeling enzymes, including the SWI/SNF ATPases SMARCA4/BRG1 and SMARCA2/BRM, ISWI enzymes SMARCA5/SNF2H and SMARCA1/SNF2L, and CHD ATPases CHD1, CHD3 and CHD4 (**Figure 19**; see included Catalog #). Each antibody is rigorously lot-validated in CUT&RUN and shows enrichment profiles consistent with expected biology (i.e. localization; see **Figure 20**). Combining biochemical EpiDyne assays (or services) with CUT&RUN profiling will improve our understanding of remodelers, opening new doors for therapeutic discovery and personalized medicine.

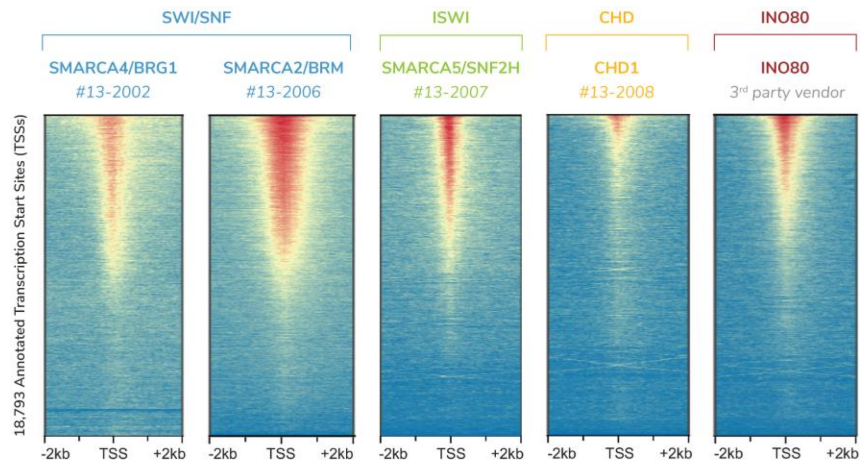


Figure 19. CUTANA™ CUT&RUN assays generate reliable profiles with high signal over background for diverse chromatin remodeling enzymes. Each reaction was performed using 500,00 K562 cells and the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048); EpiCypher antibody catalog numbers are shown above heatmaps. Heatmaps show read enrichment aligned to transcription start sites (TSS), ranked by peak signal intensity.

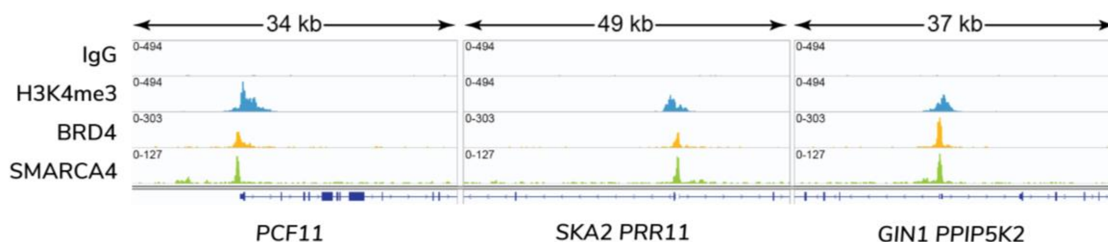


Figure 20. Representative tracks from CUT&RUN experiments show expected overlap between SMARCA4/BRG1 (EpiCypher 13-2002) and related targets, including H3K4me3 (EpiCypher 13-0041; denotes TSS) and BRD4 (EpiCypher 13-2003; interacts with SMARCA4/BRG1). IgG is a negative control. Profiles were generated using 500,000 K562 cells and the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048).

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