Nucleosome Remodeling Assay by EpiDyne™-FRET

Fully recombinant nucleosome substrates engineered for chromatin remodeling studies

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Overview

Chromatin remodeling, or the repositioning of nucleosomes, regulates DNA access and thus gene expression and genome repair. Many ATP-dependent remodeling enzyme complexes are associated with human disease but are challenging study targets due to the requirement for nucleosome-based substrates. EpiCypher has addressed this need by developing the EpiDyne platform of fully recombinant remodeling substrates to monitor nucleosome repositioning along DNA. Here, we demonstrate the utility of EpiDyne-FRET in a homogeneous remodeling assay using **F**luorescence **R**esonance **E**nergy **T**ransfer (**FRET**) readout.

Chromatin Remodeling Enzymes As Therapeutic Targets

Aberrant nucleosome organization can severely disrupt gene expression, DNA repair and cellular differentiation, and it also plays a major role in human disorders, including cancer, inflammation, autoimmunity, schizophrenia, cardiovascular disease, and intellectual disability. Remarkably, nearly 20% of all cancers contain mutations in subunits from the SWI/SNF family of ATP-dependent chromatin remodeling complexes. These enzyme complexes regulate local genome access by 'pumping' the DNA around histone octamers, thus 'sliding' nucleosomes [1]. Recurrent somatic mutations in SWI/SNF subunits are observed in multiple cancers, supporting a driver role in tumorigenesis [2]. The mutated remodeling proteins are attractive therapeutic targets, since further compromising their ATPase activity promotes cancer cell death but spares normal cells [2, 3]. This phenomenon is known as synthetic lethality, and identifying inhibitors to exploit it may lead to drugs with cancer specificity [4, 5].

Chromatin Remodeling Assays Using Recombinant Mononucleosome Substrates

EpiCypher has developed EpiDyne-FRET to study the function of chromatin remodeling enzyme complexes. The nucleosome consists of a core histone octamer wrapped in ~147 bp DNA and represents the basic repeating unit of chromatin. EpiDyne-FRET is comprised of a terminally positioned histone octamer (H2A-T120C*Cy5) wrapped in 5' Cy3-labelled DNA [8] (Figure 1). In its assembled start position, Cy3-Cy5 FRET is at maximum, with nucleosome remodeling detected by loss of the Cy5 signal as the histone octamer is relocated (towards the template DNA 3' end) or ejected.

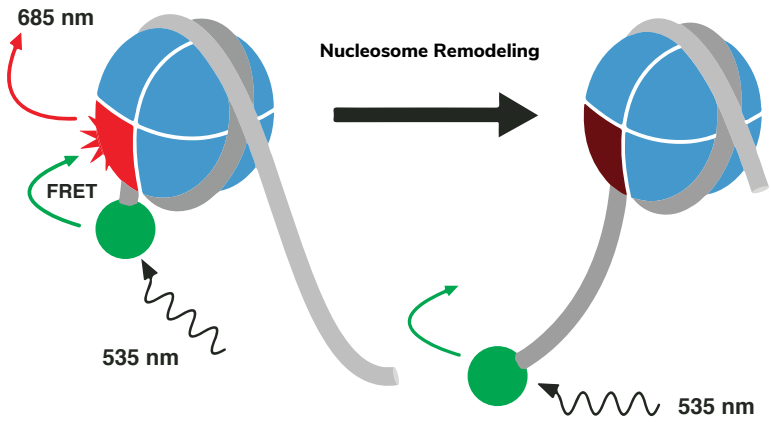

FIGURE 1: EPIDYNE-FRET NUCLEOSOME REMODELING SUBSTRATES *consist of a Cy5-labeled human histone octamer (H2A T120C-Cy5; shown as red section of octamer) wrapped by 5' Cy3-labeled DNA (217bp; green ball) comprising a terminally nucleosome positioning sequence (147bp Widom 601 [7]) adjacent to a TGGA-repeat region refractory to nucleosome assembly [8]. In its assembled starting state, Cy3-Cy5 FRET is at a maximum. The activity of an ATP-dependent remodeler (e.g. RSC or another SWI/SNF ATPase) is detected by a reduction in FRET signal as the Cy3-labeled DNA 5' end is moved away from the Cy5-labeled octamer. EpiDyne-FRET is a one-step no-wash method immediately compatible with HTS applications.*

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Nucleosome Remodeling Assay by EpiDyne™-FRET

TECHNICAL NOTE #106

Fully recombinant nucleosome substrates engineered for chromatin remodeling studies

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Reagents and Materials Required for the Assay [see Note (i)]

- Yeast RSC [9] (substrate also compatible with dACF [ACF-ISWI], dNoRC [Toutatis-ISWI] and human SMARCA2/4 (BRG1/BRM); data not shown)
- EpiDyne-FRET nucleosome remodeling substrate (EpiCypher Catalog No. 16-4201)
- Assay Buffer (20 mM Tris pH 7.5, 50 mM KCl, 3 mM MgCl2 and 0.1 mg/mL BSA (Sigma Catalog No. A3059)
- ATP (Invitrogen Catalog No. PV3227)
- Optional: ATPγS (a non-hydrolyzable form of ATP)
- Corning 3820 384-well assay plate (Fisher Catalog No. 07-200-891)
- 16 channel multipipetter for 384-well plates
- 384-well Fluorescence microplate reader capable of Cy3/Cy5-FRET detection (e.g. Envision, Perkin Elmer)

Standard Protocol

1. Determine the amount of RSC (10 nM final), EpiDyne-FRET (20 nM final), and ATP (2 mM final) needed - based on the intended time points / replicates (triplicate shown) [see Notes (ii & iii)].
2. Prepare reaction components (room temp).
   i. 2x Enzyme/Substrate solution (20 nM RSC + 40 nM EpiDyne-FRET substrate in 2x Assay Buffer)
   ii. 2x ATP +/- inhibitor solution(s) (4 mM ATP in ddH2O + inhibitors [e.g. ATPγS] as required)
3. Add 5 µL of 2x Enzyme/Substrate solution to the microplate.
4. Initiate reactions by adding 5 µL of the 2x ATP +/- inhibitor solution.
5. At appropriate timepoints read in a 384-well plate reader capable of Cy3 (excitation-531 nm / emission-579 nm) / Cy5 (emission-685 nm) detection.
6. Data is expressed as the ratio of the raw Cy3 and Cy5 emission signals at each time point [8].

NOTES

(i) Store protein aliquots at -80°C and avoid freeze/thaw.

(ii) For assay development it is advised to titrate the concentration of multiple reaction components, including enzyme, substrate, and ATP.

(iii) Since data is expressed as Cy3-Cy5 emission it is important not to add excess substrate to these reactions.

(iv) Reactions can begin rapidly. If setting up a large plate without an auto-injector there is no capability of a T0 for each condition.

References

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Acknowledgments

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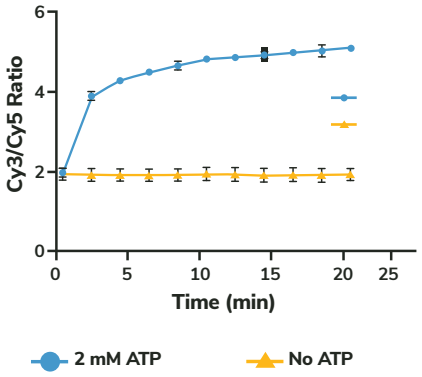

FIGURE 2: RSC/ATP-DEPENDENT NUCLEOSOME REMODELING REACTION.
EpiDyne-FRET nucleosomes (20 nM) were incubated with RSC chromatin remodeler (10 nM) in the presence or absence of 2 mM ATP (conditions previously determined as optimal for this enzyme: data not shown). Upon ATP addition, reactions were read in an Envision Multi-label plate reader at times indicated. Data is presented as the mean±SD of the Cy3/Cy5 ratio (N=6) [see Note (iv)].

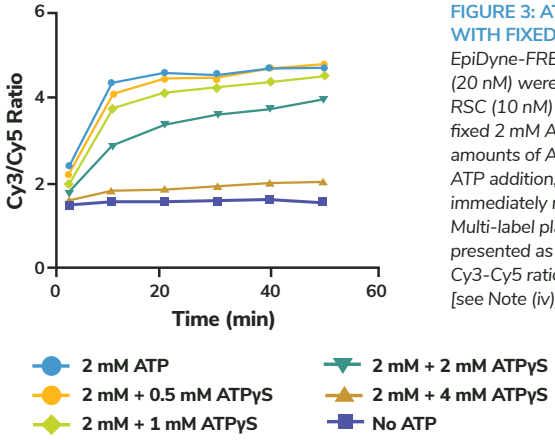

FIGURE 3: ATPγS TITRATION WITH FIXED ATP.
EpiDyne-FRET nucleosomes (20 nM) were incubated with RSC (10 nM) in the presence of fixed 2 mM ATP with increasing amounts of ATPγS. Upon ATP addition, reactions were immediately read in an Envision Multi-label plate reader. Data is presented as the mean of the Cy3-Cy5 ratio (N=2) [see Note (iv)].

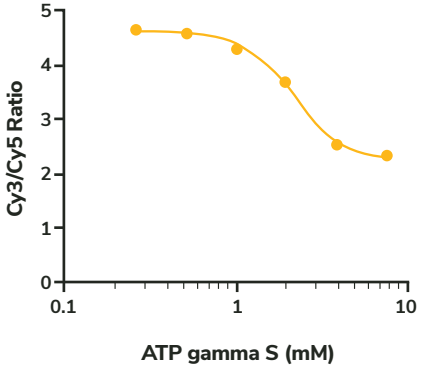

FIGURE 4: IC50 CALCULATION FOR ATPγS.
Data from Figure 3 (20-minute time point) were analyzed to determine the IC50 value for ATPγS (2.29 mM). Data is presented as the mean of the Cy3-Cy5 ratio vs. the ATPγS concentration.

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