

EpiDyne®-FRET Nucleosome Remodeling Assay Substrate



EpiCypher®

Catalog No 16-4201

Lot No 22046006-01

Pack Size 50 µg

Product Description:

This product provides a high throughput fluorescent readout of nucleosome remodeling when paired with enzymatically active remodeling complexes, such as SMARCA4, SMARCA2, and ACF (EpiCypher 15-1014, 15-1015, 15-1013). The EpiDyne®-FRET mononucleosome is assembled from recombinant human histones expressed in *E. coli* (two each of histones H2A-Cy5, H2B, H3.2 and H4; accession numbers: H2A-P04908; H2B-O60814; H3.2-Q71D13; H4-P62805) wrapped with a 212 base-pair DNA template bearing a 5' Cy3, the Widom 601 nucleosome positioning element [1], and a DpnII restriction enzyme motif (GATC) that is exposed upon remodeling. H2A has a Thr to Cys substitution at residue 120, where Cy5 is conjugated. H3.2 has a Cys to Ala substitution at residue 110.

Formulation:

Purified recombinant mononucleosomes, (21.8 µg protein weight, 50 µg DNA+protein) in 44.2 µL of 10 mM Tris-HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol. MW = 240,650 Da. Molarity = 4.7 µM.

Storage and Stability:

Stable for six (6) months at -80°C from date of receipt. For best results, aliquot and avoid multiple freeze/thaws.

Application Notes:

This product is a template for nucleosome remodeling assays using Cy3/Cy5 FRET or using the restriction enzyme DpnII to determine accessibility of GATC, which is masked in its native configuration (prior to remodeling).

DNA Sequence:

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Cy3_CATCAGAATCCCGGTGCCGAGGCCGATCAATTGGTCGTAGACAGCTCT
AGCACCCTTAAACGCACGTACGCGCTGTCCCGCGTTTTAACCGCCAAGG
GGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCGATGATGAT
GGATAGATGGATGATGGATGGATGGATGGATGGATGGATGAATAGATGGATGG
ATGAAGCTT
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References:

[1] Lowary & Widom *J Mol. Biol.* (1998) PMID: 9514715

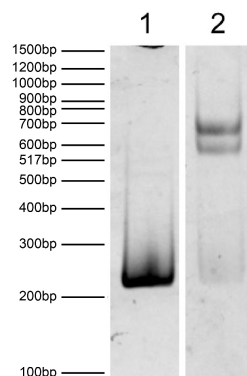


Figure 1: DNA gel data. EpiDyne-FRET nucleosome resolved via native PAGE gel and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA (100 ng). **Lane 2:** Intact EpiDyne-FRET nucleosomes (400 ng).

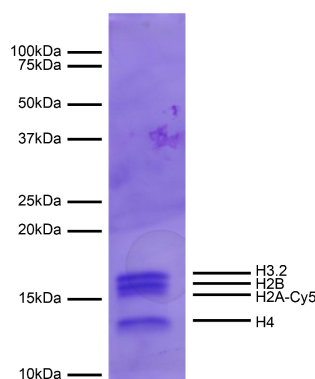


Figure 2: Protein gel data. Coomassie stained PAGE gel of proteins in EpiDyne-FRET nucleosome (1 µg) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A-Cy5, H2B, H3.2, and H4) are indicated.

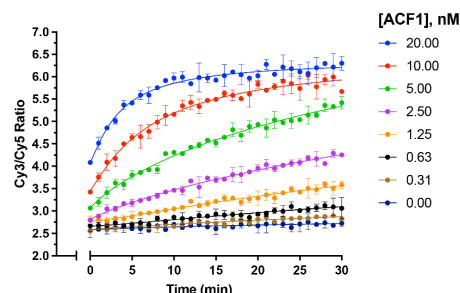


Figure 3: Nucleosome remodeling data. ACF1/ATP-dependent nucleosome remodeling reaction. EpiDyne-FRET nucleosomes (15 nM) were incubated with ACF1 chromatin remodeler (EpiCypher 15-1013; indicated concentrations) in the presence of 1 mM ATP. Upon the addition of ATP, reactions were immediately read in an Envision Multilabel plate reader (PerkinElmer). Data are presented as the mean of the Cy3/Cy5 ratio (N=2).