

# EpiDyne®-FRET Nucleosome Remodeling Assay Substrate



**Catalog No** 16-4201

**Lot No** 22046006-01

**Pack Size** 50 µg

# EpiCypher®

## 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-Q71DI3; 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:

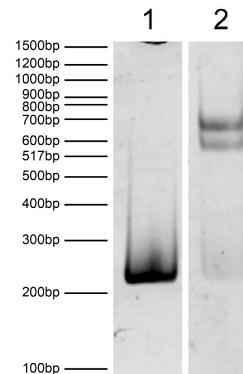
This product is a template for nucleosome remodeling assays using Cy3/Cy5 FRET or using the restriction enzyme DpnII to determine accessibility of GATC, which is masked in its native configuration (prior to remodeling).

## DNA Sequence:

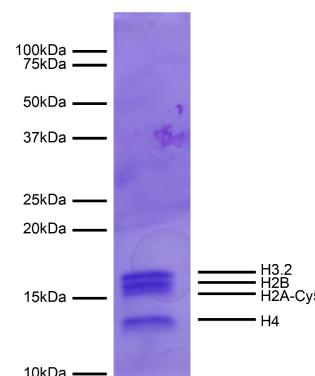
Cy3-CATCAGAACCCGGTGCCGAGGCCGATCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACCGCTGTCCCCCGGTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATACATCGATGATGATGGATAGATGGATGATGGATGGATGATGGATGAATAGATGGATGGATGGATGAAGCTT

## References:

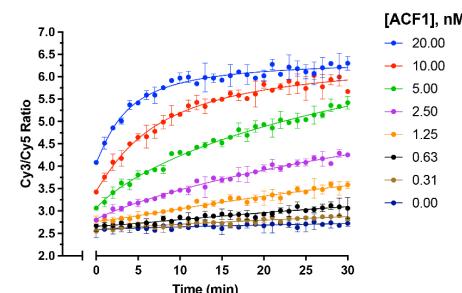
[1] Lowary & Widom J Mol. Biol. (1998) PMID: 9514715



**Figure 1: DNA gel data.** EpiDyne-FRET nucleosomes 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).