EpiDyne®-FRET Nucleosome Remodeling Assay Substrate

Catalog No	16-4201	**Species**	Human
Lot No	22046006-03	**Source**	*E. coli* & synthetic DNA
Pack Size	50 µg	**Tag**	Cy3, Cy5
Concentration	4.7 µM	**MW**	240,650 Da

DESCRIPTION

This product provides a high throughput fluorescent readout of nucleosome remodeling when paired with remodeling enzymes, such as SMARCA4, SMARCA2, and SMARCA5 (EpiCypher 15-1014, 15-1015, and 15-1024). 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) 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.

TECHNICAL INFORMATION

Storage	Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid freeze/thaws.
Formulation	Purified recombinant mononucleosomes at 1.13 mg/mL in 44.2 µL of 10 mM Tris-HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol (21.8 µg protein, 50 µg DNA + protein).

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_CATCAGAATCCCGGTGCCGAGGCC**GATC**AATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTC
CCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCGATGATGATGGATAGAT
GGATGATGGATGGATGGATGATGATGGATGAATAGATGGATGGATGAAGCTT

GENE & PROTEIN INFORMATION

UniProt ID	H2A - P04908 (alt. names: H2A type 1-B/E, H2A.2, H2A/a, H2A/m) H2B - O60814 (alt. names: H2B K, HIRA-interacting protein 1) H3.2 - Q71DI3 H4 - P62805

REFERENCES

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

VALIDATION DATA

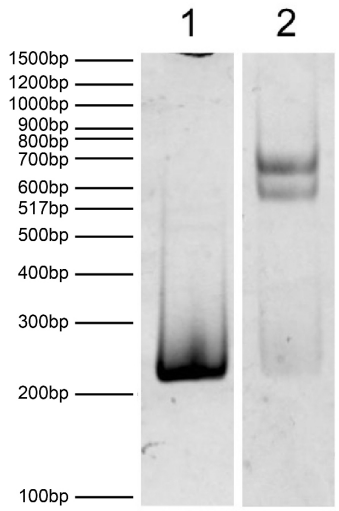

FIGURE 1 **DNA gel data.** EpiDyne-FRET nucleosome resolved via native PAGE and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA (100 ng). **Lane 2:** Intact EpiDyne-FRET nucleosomes (400 ng).

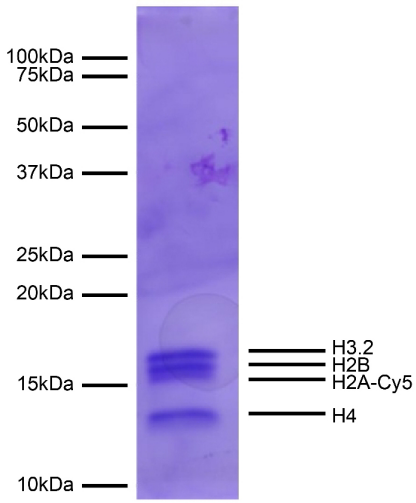

FIGURE 2 **Protein gel data.** Coomassie stained PAGE gel of proteins in EpiDyne-FRET nucleosome (1 µg) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A-Cy5, H2B, H3.2, and H4) are indicated.

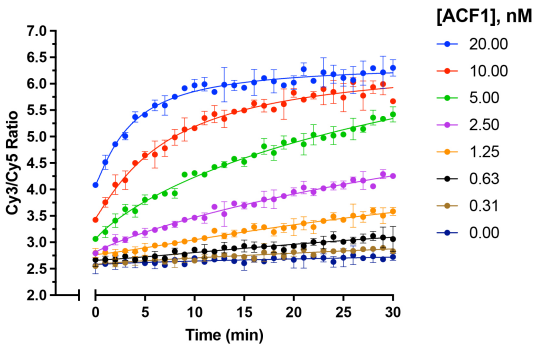

FIGURE 3 **Nucleosome remodeling data.** ACF1/ATP-dependent nucleosome remodeling reaction. EpiDyne-FRET nucleosomes (15 nM) were incubated with ACF1 chromatin remodeler at indicated concentrations in the presence of 1 mM ATP. Upon the addition of ATP, reactions were immediately read in an Envision Multilabel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