

# EpiDyne™-FRET Nucleosome Remodeling Assay Substrate

Catalog No. 16-4201  
Lot No. 17166001  
Pack Size 50 µg



## Product Description:

Mononucleosomes 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 207 base-pair 5' Cy3-conjugated DNA sequence that includes the Widom 601 nucleosome positioning element, with an added GATC motif. H2A has a Thr to Cys substitution at residue 120, the cysteine where Cy5 is conjugated. H3.2 has a Cys to Ala substitution at residue 110.

## Formulation:

Purified recombinant mononucleosomes, 50 µg at 1.37mg/ml (DNA + protein weight) in 37 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 = 5.46 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:

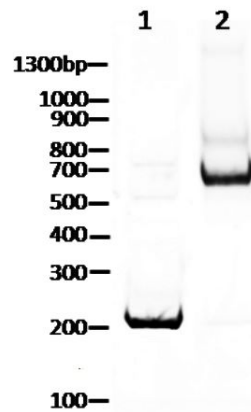
This product is a template for nucleosome remodeling assays using Cy3/Cy5 FRET, or using the restriction enzyme DpnII to determine accessibility of GATC which is masked in its native configuration (prior to remodeling).

### DNA Sequence:

**Cy3**\_CATCAGAATCCCGGTGCCGAGGCC**GATC**AATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCGCGTTTAAACGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCGATGATGATGGATAGATGGATGATGGATGGATGGATGATGGATGAATAGATGGATGGATGAAGCTT

## References:

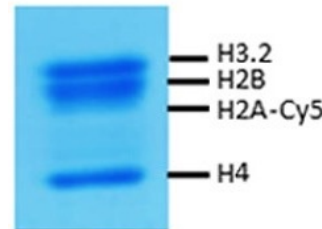
Shahian T and GJ Narlikar (2012). *Methods Mol Biol* 833: 337-349.



**DNA Gel Data:** Free DNA and Mononucleosomes run on a 6% polyacrylamide gel and stained with ethidium bromide to visualize DNA.

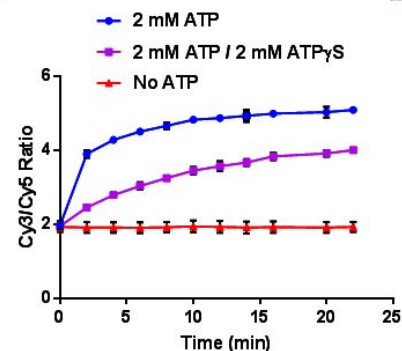
**Lane 1:** Free DNA (Cy3-ST601-GATC1 DNA, 200 ng).

**Lane 2:** Intact EpiDyne-FRET nucleosomes (370 ng).



**Protein Gel Data:** Coomassie stained PAGE gel of proteins in EpiDyne™ Nucleosome Remodeling Assay Substrate ST601-GATC1 (FRET) to demonstrate histone purity. Sizes of the histones are indicated.

N.B. Cy5 conjugation decreases mobility of H2A.



**Nucleosome Remodeling Data:** RSC/ATP-dependent nucleosome remodeling reaction. EpiDyne-FRET nucleosomes (20 nM) were incubated with RSC chromatin remodeler in the presence or absence of 2 mM ATP/ATPγS. Upon the addition of ATP, reactions were immediately read in an Envision Multilabel plate reader (PerkinElmer). Data are presented as the mean ±SD of the Cy3/Cy5 ratio (N=6).

This product is for *in vitro* research use only and is not intended for use in humans or animals.