EpiCypher®

EpiDyne® Nucleosome Remodeling Assay Substrate ST601-GATC1, Biotinylated

Catalog No	16-4111	**Species**	Human
Lot No	22173004-08	**Source**	*E.coli* & synthetic DNA
Pack Size	50 µg	**Tag**	Biotinylated
Concentration	4.1 µM	**MW**	243,380 Da

DESCRIPTION

EpiDyne® Nucleosome Remodeling Assay Substrate ST601-GATC1 consists of 217 base pairs of DNA wrapped around an octamer of core histone proteins (two each of H2A, H2B, H3.1, and H4) to form a nucleosome, the basic repeating unit of chromatin. The 217 base pair DNA sequence includes an upstream 6 bp linker, the 145 bp ST601 nucleosome positioning sequence, identified by Lowary and Widom [1], and a downstream 66 bp linker (hereby named 6-N-66 nucleosome). One copy of the 'GATC' DNA motif is embedded within the ST601 sequence and there is a 5' biotin-TEG group. The terminally positioned nucleosome is a suitable target for recognition and substrate for remodeling reactions by various chromatin interacting proteins.

TECHNICAL INFORMATION

Storage	Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid freeze/thaws.
Formulation	0.998 mg/mL in µL of 10 mM Tris-HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol. (DNA + protein weight).

APPLICATION NOTES

This product is a substrate for nucleosome remodeling assays that can monitor the accessibility of the GATC site (in bold, below). The GATC motif is masked by the nucleosome's octamer core in its native configuration and exposed to enable access by enzymes such as DpnII or dam methyltransferase after remodeling.

DNA SEQUENCE

GAATTCATCAGAATCCCGGTGCCGAGGCC**GATC**AATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGT
CCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCGATGATGATGGATAGA
TGGATGATGGATGGATGGATGATGATGGATGAATAGATGGATGGATGAAGCTT

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) H3.1 - P68431 (alt. names: H3, H3/a, H3/b, H3/c, H3/d) H4 - P62805

REFERENCES

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

VALIDATION DATA

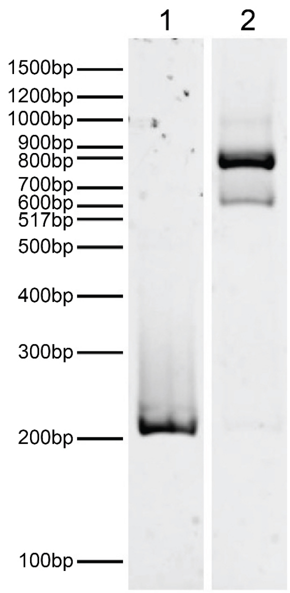

FIGURE 1: DNA gel data. ST601-GATC1 6-N-66 nucleosomes resolved via native PAGE gel and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA (EpiCypher 18-4101; 100 ng). **Lane 2:** Intact ST601-GATC1 6-N-66 nucleosomes (400 ng).

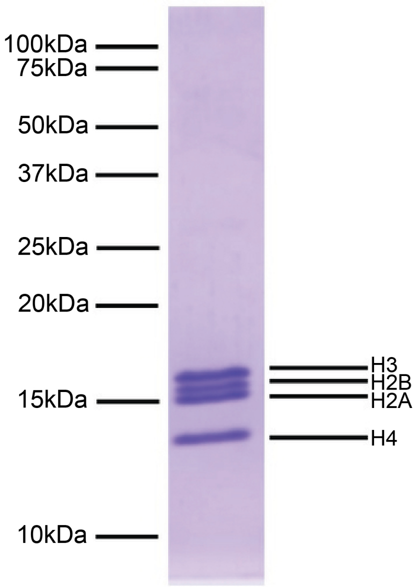

FIGURE 2: Protein gel data. Coomassie stained SDS-PAGE gel of proteins in ST601-GATC1 6-N-66 nucleosomes to demonstrate the purity of histones in the preparation (1 µg). Sizes of molecular weight markers and positions of the core histones (H2A, H2B, H3 and H4) are indicated.

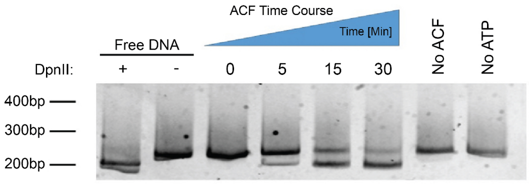

FIGURE 3: Nucleosome remodeling data. ACF/ATP-dependent nucleosome remodeling reaction in the presence of DpnII restriction enzyme. **Lanes 1 & 2:** Free DNA (EpiCypher 18-4101; 100 ng) with or without 50U of DpnII to show the migration range of the assay. **Lanes 3 - 8:** ST601-GATC1 6-N-66 nucleosomes (200 nM) incubated with 20 nM ACF for up to 30 minutes in the presence of 1 mM ATP and 50U of DpnII. Samples were quenched at specified intervals with Proteinase K, EDTA, and SDS, and resolved via 8% native PAGE.