

EpiDyne[®] Nucleosome Remodeling Assay

Substrate ST601-GATC1,2, 50-N-66, Biotin



EpiCypher[®]

Catalog No 16-4115

Lot No 21195002-02

Pack Size 50 µg

Product Description:

Mononucleosomes assembled from recombinant human histones expressed in *E. coli* (two each of histones H2A, H2B, H3 and H4. Accession numbers: H2A-P04908; H2B-O60814; H3.1-P68431; H4-P62805) wrapped by a 263 base pair DNA sequence that includes the 601 positioning sequence, identified by Lowary and Widom [1], with two added GATC sequences and a 5' biotin-TEG group. The centrally located 147 bp 601 sequence has up- and downstream linkers (50 & 66 bp each) to accommodate recognition and remodeling.

Formulation:

Purified recombinant mononucleosomes (50 µg DNA + protein weight) in 21 µL of 10 mM Tris-HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol. MW = 271,083.4 Da. Molarity = 8.78 µM.

Storage and Stability:

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

Application Notes:

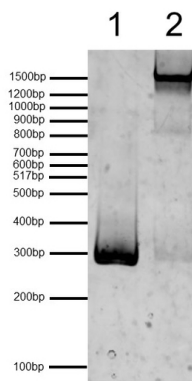
This product is a template for nucleosome remodeling assays using the restriction enzyme DpnII to determine accessibility of GATCs (in bold, below), which is masked in its native configuration and exposed after remodeling.

DNA Sequence:

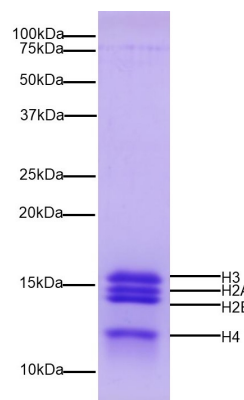
GAACCAATGGGACCATGCTTCACACCGATATCATCGCTTATGTGTTGAATTC
ATCAGAATCCCGGTGCCGAGGCC**GATCA**ATT**GATC**GTAGACAGCTCTAGCA
CCGCTTAAACGCACGTACGCGCTGTCCCGCGTTTTAACCGCCAAGGGGAT
TACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCGATGATGATGGAT
AGATGGATGATGGATGGATGGATGATGATGGATGAATAGATGGATGGATGA
AGCTT

References:

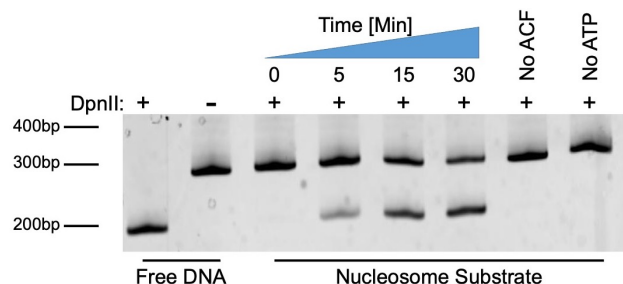
[1] Lowary PT and Widom J (1998) *J Mol Biol* 276:19-42.



DNA Gel Data: ST601-GATC1,2 50-N-66 nucleosomes resolved via native PAGE gel and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA (EpiCypher 18-4115; 100 ng). **Lane 2:** Intact ST601-GATC1,2 50-N-66 nucleosomes (400 ng).



Protein Gel Data: Coomassie stained PAGE gel of proteins in ST601-GATC1,2 50-N-66 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.



Nucleosome Remodeling Data: ACF/ATP-dependent nucleosome remodeling reaction in the presence of DpnII restriction enzyme. **Lanes 1 & 2:** Free DNA (EpiCypher 18-4115; 100 ng) with or without 50U of DpnII to show the migration range of the assay. **Lanes 3 - 8:** ST601-GATC1,2 50-N-66 nucleosomes (200 nM) incubated with 20 nM ACF for up to 30 minutes in the presence of 2 mM ATP and 50U of DpnII. Samples were quenched at specified intervals with Proteinase K and resolved via 8% native PAGE