

Acidic Patch Mutant H2BE105A,E113A Recombinant Nucleosome with Linker DNA, Biotinylated

Catalog No	16-2031	Species	Human
Lot No	23346001-02	Source	<i>E. coli</i> & synthetic DNA
Pack Size	50 µg	Tag	Biotinylated
Concentration	5.2 µM	MW	231,649 Da

DESCRIPTION

The acidic patch is a negatively charged region of the nucleosome surface that serves as a conserved interaction hub for neighboring nucleosomes and nucleosome binding proteins, often via salt bridges with arginine anchors [1]. The acidic patch plays a critical role in chromatin condensation and chromatin remodeling [1-3]. Recombinant mononucleosomes containing acidic patch mutations can be used to study the biological functions of the acidic patch.

H2BE105A,E113A Recombinant Nucleosome with Linker DNA consists of 199 base pairs of DNA wrapped around an octamer core of histone proteins (two each of H2A, H2B, H3.1, and H4) to form a nucleosome, the basic repeating unit of chromatin. The 5' biotin-TEG DNA consists of a core 147 bp 601 nucleosome assembly sequence [4] flanked by 26 bp linker sequences as underlined below. Histone H2B contains a glutamate-to-alanine (E-to-A) substitution at positions 105 and 113 (H2BE105A, E113A). H2BE105 and H2BE113 both reside in H2B alphaC helix extension and are associated with nucleosome binding factors such as H4-N-terminal tail, LANA, RCC1, and HMGN2 [1]. H2BE105A,E113A disrupts binding with SMARCB1/BAF47, a subunit of the SWI/SNF (BAF) family of chromatin remodeling complexes. These complexes serve a critical role in cell division, cell and tissue differentiation, and development, and SWI/SNF complex malfunction has been linked to over 20% of human cancers [5].

TECHNICAL INFORMATION

Storage	Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid freeze/thaws.
Formulation	1.2 mg/mL mononucleosome in 41.6 µL 10 mM Tris-HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol. (27.3 µg protein, 50 µg DNA + protein)

APPLICATION NOTES

H2BE105A,E113A mononucleosome is highly purified and suitable for a variety of applications to test the effect of acidic patch mutation on enzymatic activity or chromatin binding. The biotinylated DNA enables affinity binding applications. For a corresponding unmodified control, we recommend EpiCypher 16-2044.

DNA SEQUENCE

5'-Bio-TEG

GGACCCTATACGCGGCCCGCGAATTCCTGGAGAATCCCGGTCTGCAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCG
CTTAAACGCACGTACGCGCTGTCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATA
TACATCCTGTGGATCCGCGGTTCGCGAACAGCGACC3'

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
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VALIDATION DATA

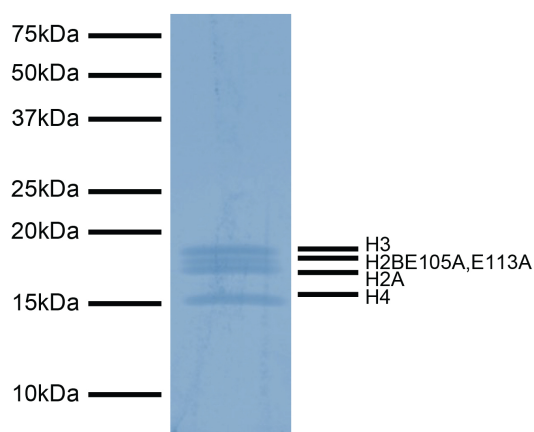


FIGURE 1 Protein gel data. Coomassie stained SDS-PAGE gel of proteins in H2BE105A,E113A recombinant nucleosome (1 µg) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2BE105A,E113A, H3 and H4) are indicated.

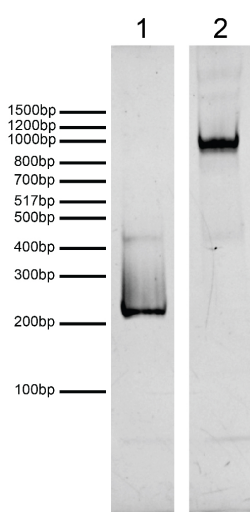


FIGURE 2 DNA gel data. H2BE105A,E113A recombinant nucleosome resolved by native PAGE and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA (EpiCypher 18-2044; 100 ng). Biotinylated DNA can dimerize (band at ~400 bp). **Lane 2:** Intact H2BE105A,E113A recombinant nucleosomes (400 ng).

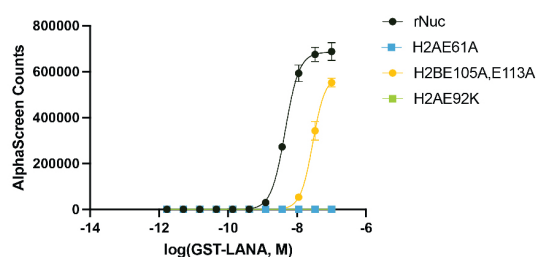


FIGURE 3 Functional binding assay. The presence of the acidic patch mutations disrupts LANA peptide binding to recombinant nucleosomes (WT control, EpiCypher 16-2044; H2AE61A, EpiCypher 16-2029; H2AE92K, EpiCypher 16-2030; H2BE105A,E113A, EpiCypher 16-2031). The binding of GST-tagged LANA peptide to biotinylated recombinant nucleosomes was assessed by AlphaLISA assay using Streptavidin Donor Beads and Glutathione Acceptor Beads (PerkinElmer). The presence of H2A acidic patch mutations completely blocks LANA binding, while H2B mutations cause a decrease in LANA binding affinity.

REFERENCES

- [1] Kalashnikova et al. *J. R. Soc. Interface* (2013). PMID: 23446052
- [2] Levendosky & Bowman *eLife* (2019). PMID: 31094676
- [3] Gamarra et al. *eLife* (2018). PMID: 29664398
- [4] Lowary & Widom *J. Mol. Biol.* (1998). PMID: 9514715
- [5] Valencia et al. *Cell* (2019). PMID: 31759698