Nucleosome, Recombinant Human, Acidic Patch Mutant H2BE105A, E113A Biotinylated

Catalog No	16-0031	**Species**	Human
Lot No	22217002-02	**Source**	*E. coli* & synthetic DNA
Pack Size	50 µg	**Tag**	Biotinylated
Concentration	4.43 µM	**MW**	199,510 Da

DESCRIPTION

Recombinant mononucleosomes consist of 147 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 147 bp 601 sequence containing a 5' biotin-TEG group has high affinity for histone octamers and is useful for nucleosome assembly [1]. Histone H2B contains a glutamate-to-alanine (E-to-A) substitution at positions 105 and 113 (H2BE105A, E113A). H2BE105 and H2BE113 are among key residues forming a negatively charged region on the nucleosome surface named the "acidic patch". The acidic patch is a conserved interaction hub for neighboring nucleosomes and nucleosome binding proteins, often via salt bridges with arginine anchors, and is functionally critical in chromatin condensation and chromatin remodeling [2-4]. H2B 105 and E113 both reside in H2B alphaC extension and are associated with nucleosome binding factors such as histone H4 N-terminal tail, LANA, RCC1, HMGN2, and SMARCB1 [2,5].

TECHNICAL INFORMATION

Storage	Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid freeze/thaws.
Formulation	0.884 mg/mL mononucleosome in 56.5 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 mutations on enzymatic activity or chromatin binding. The biotinylated DNA enables affinity binding applications.

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
[2] Kalashnikova et al. *Soc. Interface* (2013). PMID: 23446052
[3] Levendosky & Bowman *eLife* (2019). PMID: 31094676
[4] Gamarra et al. *eLife* (2018). PMID: 29664398
[5] Valencia et al. *Cell* (2019). PMID: 31759698

VALIDATION DATA

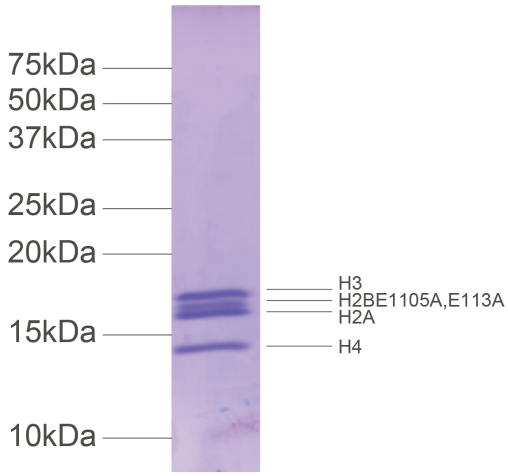

FIGURE 1 **Protein gel data.** Coomassie stained PAGE gel of proteins in H2BE105A, E113A mononucleosome (1 µg) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2BE105A,E113A, H3 and H4) are indicated.

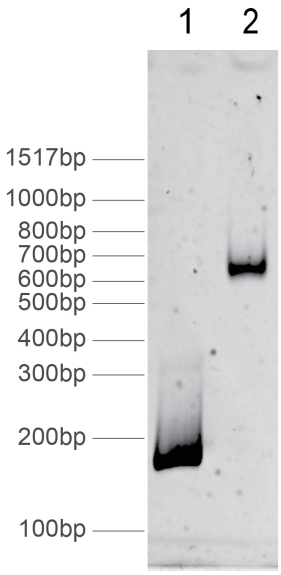

FIGURE 2 **DNA gel data.** H2BE105A,E113A mononucleosome resolved by native PAGE and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA (EpiCypher 18-0005; 100 ng). **Lane 2:** Intact H2BE105A,113A mononucleosomes (400 ng).

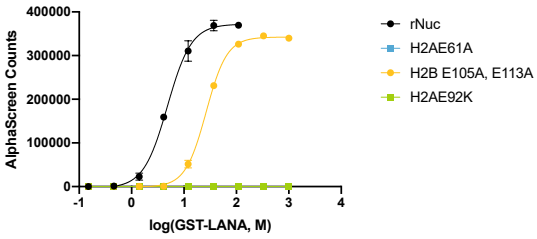

FIGURE 3 **Functional binding assay.** The presence of acidic patch mutations disrupts LANA peptide binding to recombinant nucleosomes (WT control, EpiCypher 16-0006; H2AE61A, EpiCypher 16-0029; H2AE92K, EpiCypher 16-0030; H2BE105A,E113A, EpiCypher 16-0031). The binding of GST-tagged LANA peptide to 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.