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

Catalog No. 16-0031
Lot No. 21119001-01
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 147 base pairs of 601 positioning sequence DNA [1] containing a 5' biotin-TEG group. *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 E105 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].

Formulation:

H2BE105A,E113A mononucleosomes (27.3 µg protein weight, 50 µg DNA+protein) in 48.5 µL 10 mM Tris HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol. Molarity = 5.16 µM. MW = 199,627.7 Da.

Storage and Stability:

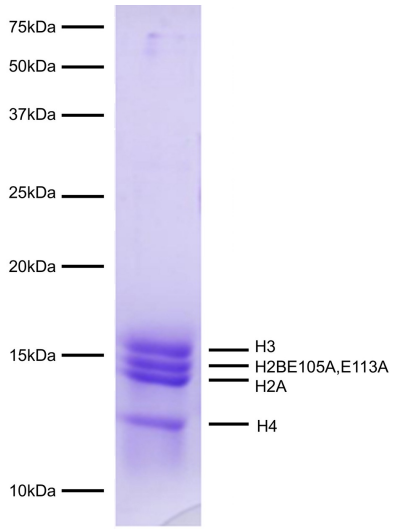
Stable for six (6) months at -80°C from date of receipt. For best results, aliquot and avoid multiple freeze/thaws.

Application Notes:

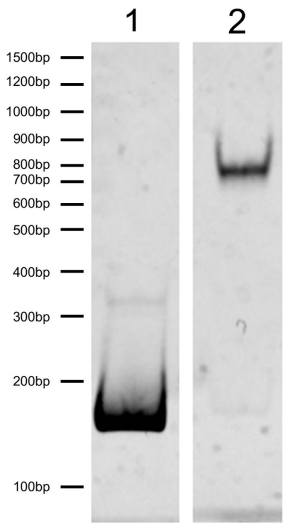
H2BE105A,E113A mononucleosomes are highly purified and suitable for a variety of applications to test the effect of acidic patch mutations on enzymatic activity or chromatin binding. The biotinylated DNA enables affinity binding applications.

References:

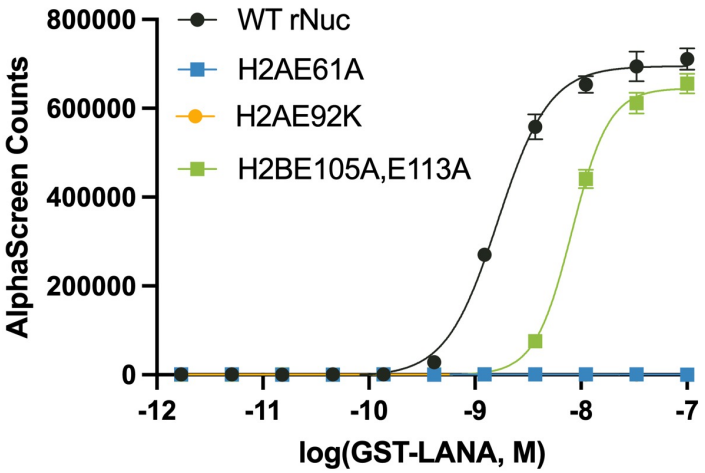
[1] Lowary PT and Widom J (1998) J. Mol. Biol. 276:19-42.
[2] Kalashnikova AA *et al.* (2013) *J. R. Soc. Interface* 10:20121022.
[3] Levendosky RF and Bowman GD (2019) *eLife* 8:e45472.
[4] Gamarra N *et al.* (2018) *eLife* 7:e35322.
[5] Valencia AM *et al.* (2019) *Cell* 179:1342-1356.



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



DNA Gel Data: H2BE105A,E113A mononucleosomes resolved by native PAGE and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA (100 ng). **Lane 2:** Intact nucleosomes (400 ng).



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 (Perkin Elmer). The presence of H2A acidic patch mutants completely blocks LANA binding, while H2B mutations cause a decrease in LANA binding affinity.