Nucleosome, Recombinant Human, Acidic Patch Mutant H2AE92K Biotinylated

Catalog No. 16-0030
Lot No. 21119001-02
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 147 base pairs of 601 positioning sequence DNA [1] containing a 5' biotin-TEG group. *Histone H2A contains a glutamate-to-lysine (E-to-K) substitution at position 92 (H2AE92K). H2AE92 is 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]. H2AE92 resides in the H2A C-terminal extension and is associated with nucleosome binding factors such as histone H4 N-terminal tail, LANA, RCC1, IL-33, SIR3, HMGN2, and remodeling ATPases [2-4]. H2AE92K disrupts binding with SMARCB1 [5].

Formulation:

H2AE92K mononucleosomes (27.3 µg protein weight, 50 µg DNA+protein) in 49.5 µL 10 mM Tris HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol. Molarity = 5.05 µM. MW = 199,839.9 Da.

Storage and Stability:

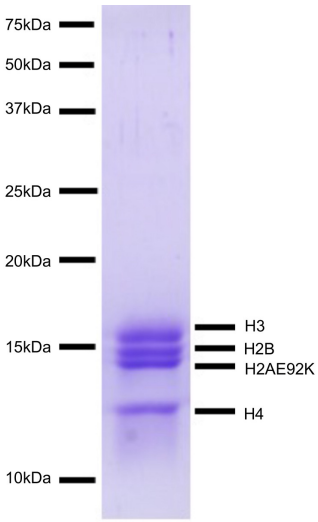
Stable for six (6) months at -80°C from date of receipt. For best results, aliquot and avoid multiple freeze/thaws.

Application Notes:

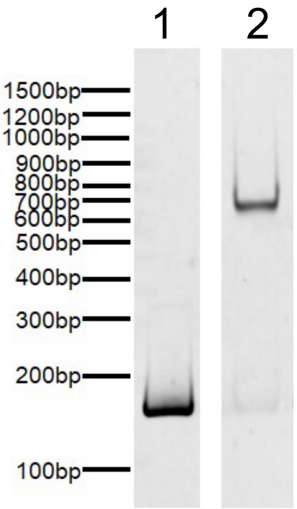
H2AE92K mononucleosomes are highly purified and suitable for a variety of applications to test the effect of acidic patch mutation on enzymatic activity or chromatin binding. The biotinylated DNA enables affinity binding applications.

References:

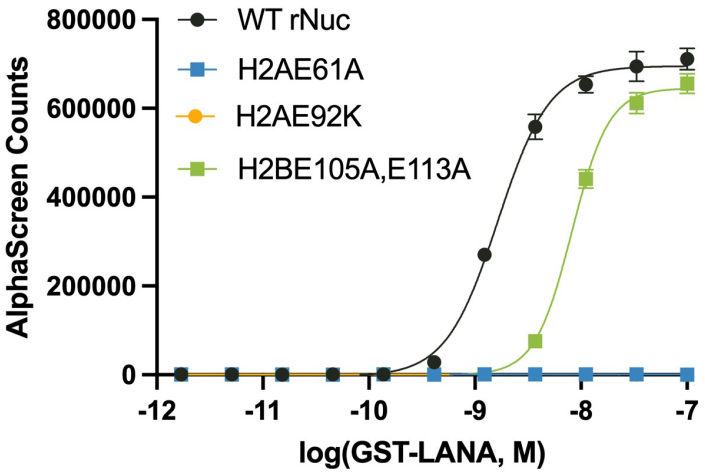
[1] Lowary PT and Widom J (1998) J. Mol. Biol. 276:19-42.
[2] Kalashnikova AA *et al.* (2013) *J. R. Soc. Interface* 10:20121022.
[3] Levendosky RF and Bowman GD (2019) *eLife* 8:e45472.
[4] Gamarra N *et al.* (2018) *eLife* 7:e35322.
[5] Valencia AM *et al.* (2019) *Cell* 179:1342-1356.



Protein Gel Data: Coomassie stained PAGE gel of proteins in H2AE92K mononucleosomes (1 µg) demonstrates the purity of the histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2AE92K, H2B, H3.1 and H4) are indicated.



DNA Gel Data: H2AE92K 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 mutations completely blocks LANA binding, while H2B mutations cause a decrease in LANA binding affinity.