

Nucleosome, Recombinant Human, Acidic Patch Mutant H2AE61A

Catalog No. 16-1029

Lot No. 21119001-06

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].

*Histone H2A contains a glutamate-to-alanine (E-to-A) substitution at position 61 (H2AE61A). H2AE61A 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]. H2AE61 resides in the alpha2 helix and forms a key salt bridge with H4K16. H2AE61 mediates chromatin binding with factors such as LANA, RCC1, IL-33, SIR3 and HMG2 [2]. H2AE61A disrupts chromatin remodeling by the ISWI remodeler SNF2h [4].

Formulation:

H2AE61A mononucleosomes (27.3 µg protein weight, 50 µg DNA+protein) in 89.7 µL 10 mM Tris HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol. Molarity = 2.8 µM. MW = 199,144.9 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:

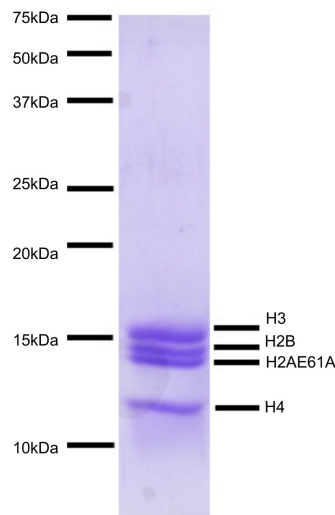
H2AE61A mononucleosomes are highly purified and suitable for a variety of applications to test the effect of acidic patch mutation on enzymatic activity or chromatin binding. See EpiCypher 16-0029 for a biotinylated version of this mutant.

References:

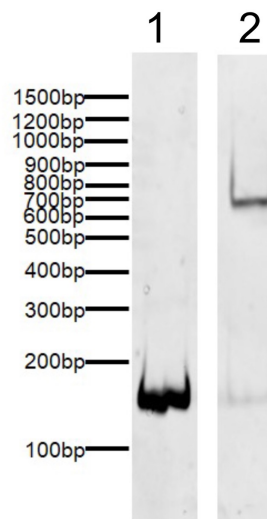
- [1] Lowary PT and Widom J (1998) *J. Mol. Biol.* 276:19-42.
- [2] Kalashnikova AA et al. (2013) *J. R. Soc. Interface* 10:20121022.
- [3] Levodosky RF and Bowman GD (2019) *eLife* 8:e45472.
- [4] Gamarra N et al. (2018) *eLife* 7:e35322.



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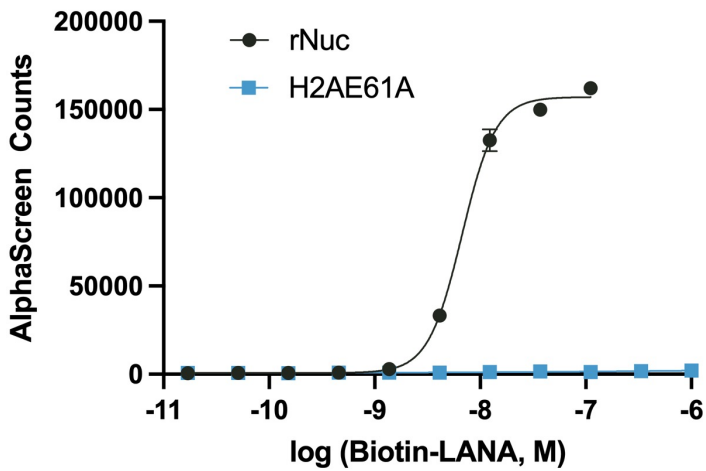


Protein Gel Data: Coomassie stained PAGE gel of proteins in H2AE61A mononucleosomes (1 µg) demonstrates the purity of the histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2AE61A, H2B, H3.1 and H4) are indicated.



DNA Gel Data: H2AE61A 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).

This product is for *in vitro* research use only and is not intended for use in humans or animals.



Functional Binding Assay: The presence of the acidic patch mutation disrupts LANA peptide binding to recombinant nucleosomes (WT rNuc control, EpiCypher 16-0009; H2AE61A, EpiCypher 16-1029). The binding of biotinylated LANA peptide to recombinant nucleosomes was assessed by AlphaLISA assay (Perkin Elmer) using Streptavidin Donor Beads, anti-Histone H3.1/3.2 antibody, and Protein A Acceptor Beads.

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