Nucleosome, Recombinant Human, Acidic Patch Mutant H2AE61A

Catalog No	16-1029	**Species**	Human
Lot No	22126002-01	**Source**	*E. coli* & synthetic DNA
Pack Size	50 µg	**Tag**	None
Concentration	4.2 µM	**MW**	199,144.9 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, identified by Lowary and Widom [1], has high affinity for histone octamers and is useful for nucleosome assembly. 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 HMGN2 [2]. H2AE61A disrupts chromatin remodeling by the ISWI remodeler SMARCA5/SNF2h [4].

TECHNICAL INFORMATION

Storage	Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid freeze/thaws.
Formulation	0.84 mg/mL mononucleosome in 59.8 uL 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

H2AE61A 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. See EpiCypher 16-0029 for a biotinylated version of this mutant.

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

VALIDATION DATA

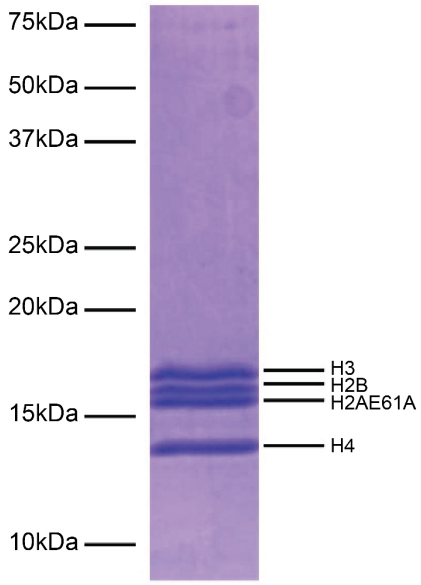

FIGURE 1 Protein gel data. Coomassie stained PAGE gel of proteins in H2AE61A mononucleosome (1 µg) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2AE61A, H2B, H3 and H4) are indicated.

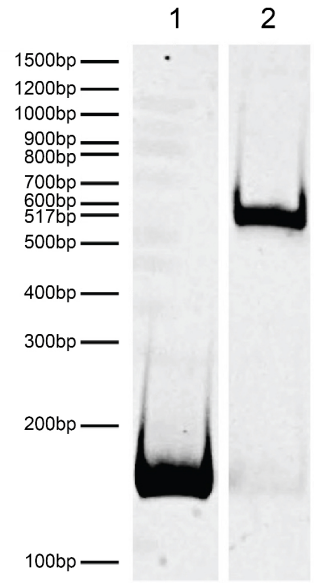

FIGURE 2 DNA gel data. H2AE61A mononucleosome resolved via native PAGE and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA (EpiCypher 18-0005; 100 ng). **Lane 2:** Intact H2AE61A mononucleosomes (400 ng).

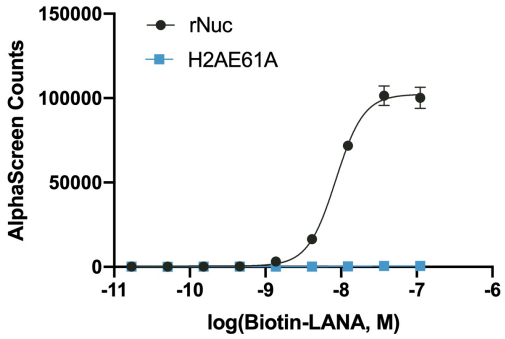

FIGURE 3 Functional binding assay. The presence of the acidic patch mutation disrupts LANA peptide binding to recombinant nucleosomes (WT rNuc control, EpiCypher 16-0009; H2AE61A, EpiCypher 16-1029). The binding of biotinylated LANA peptide to recombinant nucleosomes was assessed by AlphaLISA assay (Perkin Elmer) using Streptavidin Donor Beads, anti-Histone H3.1/3.2 antibody, and Protein A Acceptor Beads.