# Acidic Patch Mutant H2AE61A Recombinant Nucleosome with Linker DNA, Biotinylated

| | | | |
|---|---|---|---|
| **Catalog No** | 16-2029 | **Species** | Human |
| **Lot No** | 23346001-01 | **Source** | *E. coli* & synthetic DNA |
| **Pack Size** | 50 µg | **Tag** | Biotinylated |
| **Concentration** | 5.6 µM | **MW** | 231,766 Da |

## DESCRIPTION

The acidic patch is a negatively charged region of the nucleosome surface that serves as a conserved interaction hub for neighboring nucleosomes and chromatin binding proteins, often via salt bridges with arginine anchors [1]. The acidic patch plays a critical role in chromatin condensation and chromatin remodeling [1-3]. Recombinant mononucleosomes containing acidic patch mutations can be used to study the biological functions of the acidic patch.

H2AE61A Recombinant Nucleosome with Linker DNA consists of 199 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 5' biotin-TEG DNA consists of a core 147 bp 601 nucleosome assembly sequence [4] flanked by 26 bp linker sequences as underlined below. Histone H2A contains a glutamate-to-alanine (E-to-A) substitution within the acidic patch at position 61 (H2AE61A). H2AE61 resides in the alpha2 helix, forming a key salt bridge with H4K16, and mediates chromatin binding with factors such as LANA, RCC1, IL-33, Sir3, and HMGN2 [1]. H2AE61A disrupts chromatin remodeling by SMARCA5/SNF2h, an ATPase subunit of the ISWI family of chromatin remodeling complexes [2,3].

## TECHNICAL INFORMATION

| | |
|---|---|
| **Storage** | Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid freeze/thaws. |
| **Formulation** | 1.3 mg/mL mononucleosome in 38.4 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

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. The biotinylated DNA enables affinity binding applications. For a corresponding unmodified control, we recommend EpiCypher 16-2044.

## DNA SEQUENCE

5'-Bio-TEG
GGACCCTATACGCGGCCGCCGAATTCCTGGAGAATCCCGGTCTGCAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCG
CTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATA
TACATCCTGTGGATCCGCCGGTCGCGAACAGCGACC3'

## GENE & PROTEIN INFORMATION

| | |
|---|---|
| **UniProt ID** | H2A - P04908 (alt. names: H2A type 1-B/E, H2A.2, H2A/a, H2A/m)<br>H2B - O60814 (alt. names: H2B K, HIRA-interacting protein 1)<br>H3.1 - P68431 (alt. names: H3, H3/a, H3/b, H3/c, H3/d)<br>H4 - P62805 |

## VALIDATION DATA

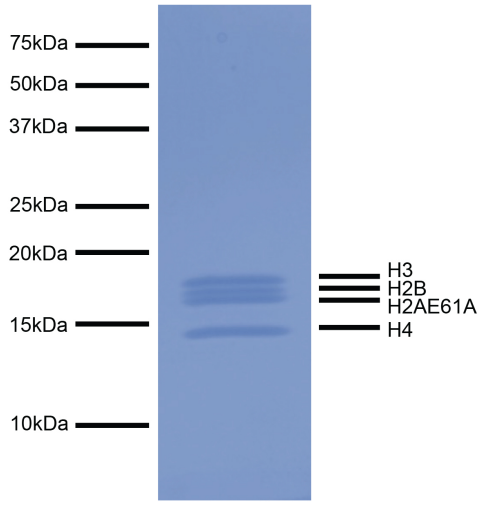


**FIGURE 1 Protein gel data.** Coomassie stained SDS-PAGE gel of proteins in H2AE61A recombinant nucleosome (1 µg) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2AE61A, H2B, H3 and H4) are indicated.

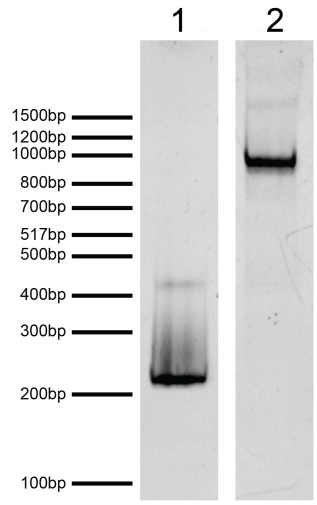


**FIGURE 2 DNA gel data.** H2AE61A recombinant nucleosome resolved by native PAGE and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA (EpiCypher 18-2044; 100 ng). Biotinylated DNA can dimerize (band at ~400 bp). **Lane 2:** Intact H2AE61A recombinant nucleosomes (400 ng).

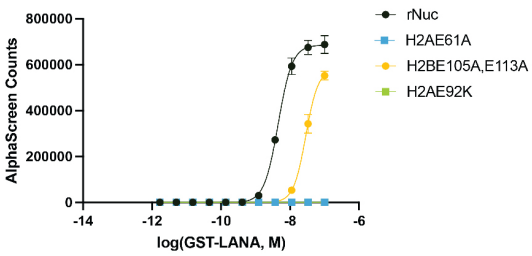


**FIGURE 3 Functional binding assay.** The presence of the acidic patch mutations disrupts LANA peptide binding to recombinant nucleosomes (WT control, EpiCypher 16-2044; H2AE61A, EpiCypher 16-2029; H2AE92K, EpiCypher 16-2030; H2BE105A,E113A, EpiCypher 16-2031). The binding of GST-tagged LANA peptide 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.

## REFERENCES

[1] Kalashnikova et al. *J. R. Soc. Interface* (2013). PMID: 23446052
[2] Levendosky & Bowman *eLife* (2019). PMID: 31094676
[3] Gamarra et al. *eLife* (2018). PMID: 29664398
[4] Lowary & Widom *J. Mol. Biol.* (1998). PMID: 9514715