Nucleosome, Recombinant Human, H3.3S31phos dNuc, Biotinylated

Catalog No	16-0389	**Species**	Human
Lot No	22202003-03	**Source**	*E. coli* & synthetic DNA
Pack Size	50 µg	**Tag**	Biotinylated
Concentration	3.9 µM	**MW**	199,867 Da

DESCRIPTION

EpiCypher dNucs™ are semi-synthetic recombinant nucleosomes, containing one or more histone post translational modifications (PTMs). Nucleosomes, the basic repeating unit of chromatin, are the natural targets of chromatin readers and modifying enzymes, providing a physiologically relevant substrate for characterization of epigenetic proteins. dNucs consist of 147 base pairs of DNA wrapped around an octamer core of histone proteins (two each of H2A, H2B, H3.3, and H4). The 147 bp 601 sequence, identified by Lowary and Widom [1], has high affinity for histone octamers and is useful for nucleosome assembly. H3.3S31phos dNuc contains phosphoserine at position 31 on histone H3.3, which has shown to be a negative regulator of KDM4B binding, and thereby preserving H3K9me3 marks [2]. The DNA in this nucleosome contains a 5'biotin-TEG group.

TECHNICAL INFORMATION

Storage	Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid freeze/thaws.
Formulation	0.78 mg/mL mononucleosome in 64.1 uL 10 mM Tris HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol (27.4 µg protein, 50 µg DNA + protein)

APPLICATION NOTES

H3.3S31phos dNuc is highly purified and suitable for a variety of applications, including use as a substrate in enzyme assays, high-throughput screening and inhibitor testing, chromatin binding studies, protein-protein interaction assays, structural studies, and in effector protein binding experiments.

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) H3.3 - P84243 H4 - P62805

REFERENCES

[1] Lowary & Widom *J. Mol. Biol.* (1998). PMID: 9514715
[2] Udugama et al. *Nucleic Acids Res.* (2022). PMID: 35451487

VALIDATION DATA

FIGURE 1: Western blot data. Western Analysis of H3.3S31phos dNuc. **Top Panel:** Unmodified (EpiCypher 16-0006; Lane 1) and H3.3S31phos nucleosomes (Lane 2) were probed with an anti-H3.3S31phos antibody and analyzed via ECL readout. Only the H3.3S31phos sample produced a detectable signal. **Bottom Panel:** Detail from Coomassie stained gel showing unmodified (Lane 1) and H3.3S31phos nucleosomes (Lane 2).

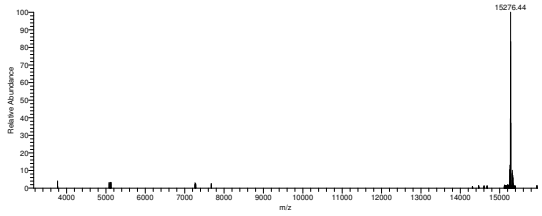

FIGURE 2: Mass spec data. Semi-synthetic H3.3S31phos histone analyzed by high resolution mass spectrometry. Expected mass = 15,276.7 Da. Determined mass = 15,276.44 Da.

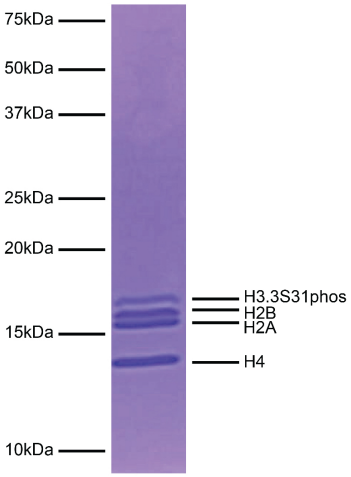

FIGURE 3: Protein gel data. Coomassie stained SDS-PAGE gel of proteins in H3.3S31phos dNuc (1 µg) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2B, H3.3S31phos and H4) are indicated.

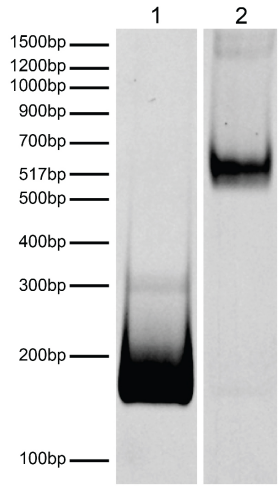

FIGURE 4: DNA gel data. H3.3S31phos dNuc resolved via native PAGE gel and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA (EpiCypher 18-0005; 100 ng). **Lane 2:** Intact H3.3S31phos nucleosomes (400 ng).