H3T3phos Recombinant Nucleosome, Biotinylated

Catalog No	16-0404	**Species**	Human
Lot No	24128001-01	**Source**	*E. coli* & synthetic DNA
Pack Size	50 µg	**Tag**	Biotinylated
Concentration	4.9 µM	**MW**	199,806 Da

DESCRIPTION

Histone phosphorylation is a post-translational modification (PTM) wherein a phosphate group is added to a histone protein, predominantly occurring on serine, threonine, and tyrosine residues. In combination with other PTMs, histone phosphorylation constitutes the "histone code," acting as a language read by proteins to regulate chromatin structure and gene expression. Histone phosphorylation is involved in chromatin remodeling and compaction associated with diverse cellular processes, including DNA damage repair, transcription regulation, cell division, and apoptosis [1]. Recombinant mononucleosomes containing phosphorylated histones can be used to study the biological functions of histone phosphorylation.

H3T3phos (histone H3 threonine 3 phosphorylation) Recombinant Nucleosome, Biotinylated consists of 147 base pairs of DNA wrapped around an octamer of core histone proteins (two each of H2A, H2B, H3.2, and H4) to form a nucleosome, the basic repeating unit of chromatin. The 147 bp 601 sequence, identified by Lowary and Widom [2], has high affinity for histone octamers and is useful for nucleosome assembly. The DNA contains a 5' biotin-TEG group. H3T3phos nucleosome contains phosphorylated threonine at position 3 on histone H3.2 and a Cys to Ala substitution at position 110. H3T3 is phosphorylated during mitosis by Haspin, a mitotic chromatin-associated kinase, and becomes highly enriched at inner centromeric regions during prometaphase and metaphase. H3T3phos is associated with chromatin segregation and binds to Survivin, a subunit of Chromosomal Passenger Complex (CPC), to recruit CPC to the centromere during mitosis [1].

TECHNICAL INFORMATION

Storage	Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid freeze/thaws.
Formulation	0.98 mg/mL mononucleosome in 51 µL 10 mM Tris HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol (27.2 µg protein, 50 µg DNA + protein).

APPLICATION NOTES

H3T3phos mononucleosome 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. For a corresponding unmodified control, we recommend EpiCypher 16-0006.

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) H3.2 - Q71DI3 H4 - P62805

REFERENCES

[1] Rossetto et al. *Epigenetics* (2012). PMID: 22948226
[2] Lowary & Widom *J. Mol. Biol.* (1998). PMID: 9514715

VALIDATION DATA

FIGURE 1 Western blot data. Western Analysis of H3T3phos nucleosome. **Top Panel:** H3T3phos (Lane 1) and unmodified (EpiCypher 16-0006; Lane 2) nucleosomes were probed with an anti-H3T3phos antibody and analyzed via enhanced chemiluminescence (ECL) readout. Only the H3T3phos sample produced a detectable signal. **Bottom Panel:** Detail from Coomassie stained gel showing H3T3phos (Lane 1) and unmodified (Lane 2) nucleosomes.

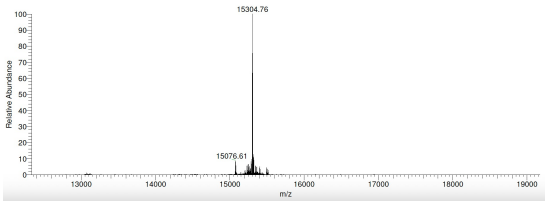

FIGURE 2 Mass spec data. Synthetic H3T3phos histone analyzed by high resolution mass spectrometry. Expected mass = 15,304.8 Da. Determined mass = 15,304.76 Da.

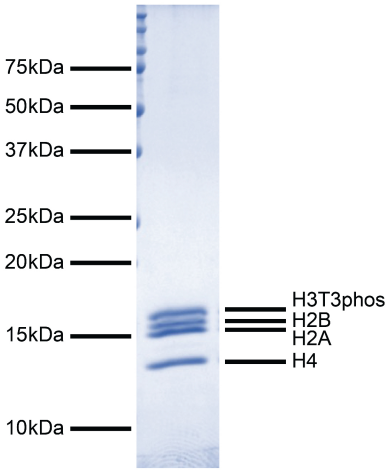

FIGURE 3 Protein gel data. Coomassie stained PAGE gel of proteins in H3T3phos nucleosome (1 µg) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2B, H3T3phos, and H4) are indicated.

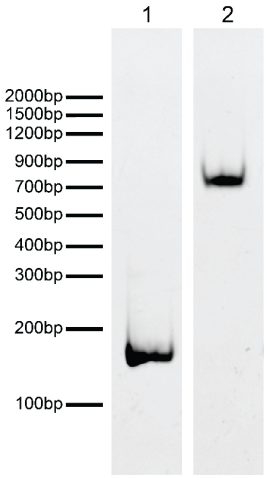

FIGURE 4 DNA gel data. H3T3phos nucleosome resolved via native PAGE and stained with ethidium bromide to visualize DNA. Both lanes are from the same gel. **Lane 1:** Free DNA (EpiCypher 18-0005; 100 ng). **Lane 2:** Intact H3T3phos nucleosomes (400 ng).