

## Heterotypic [H3K4me3 • H3K9,14,18ac] Recombinant Nucleosome, Biotinylated

<b>Catalog No</b>	16-0415	<b>Species</b>	Human
<b>Lot No</b>	24219001-85	<b>Source</b>	<i>E. coli</i> & synthetic DNA
<b>Pack Size</b>	25 µg	<b>Tag</b>	Biotinylated
<b>Concentration</b>	3.5 µM	<b>MW</b>	199,815 Da

### DESCRIPTION

Heterotypic nucleosomes, also referred to as “asymmetric nucleosomes,” contain sister histones with distinct histone variants and/or post-translational modifications (PTMs). In homotypic nucleosomes, or “symmetric nucleosomes,” each pair of sister histones bears the same PTM, set of PTMs, or histone variant. Histone-modifying enzymes, chromatin remodelers, and histone chaperones differentially modify sister histones or exchange unique histone variants to form heterotypic nucleosomes. Heterotypic nucleosomes have been found at promoters of developmental genes in undifferentiated embryonic stem cells and transcription start sites (TSSs) of approximately half of the genes in budding yeast [1]. Heterotypic nucleosomes represent an additional layer of the histone code, acting as substrates for multivalent reader proteins, participating in PTM crosstalk mechanisms, and influencing reader protein binding affinity through varying local target concentration. Recombinant heterotypic nucleosomes are useful for studying chromatin dynamics and transcriptional regulation.

Heterotypic [H3K4me3 • H3K9,14,18ac] Recombinant Nucleosome, Biotinylated is a fully defined semi-synthetic nucleosome containing combinatorial PTMs in both *cis* and *trans* whereby one histone H3 is trimethylated at lysine four while the other is acetylated at lysines 9, 14, and 18. [H3K4me3 • H3K9,14,18ac] nucleosome consists of 147 base pairs of 601 sequence DNA [2] 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 DNA contains a 5' biotin-TEG group, and histone H3.2 has a Cys to Ala substitution at position 110. H3 hyperacetylation in *cis* (e.g. H3K9,14,18ac) increases tail accessibility for H3K4 methyl writers such as the methyltransferase MLL1, emphasizing the role of PTM crosstalk and heterotypic nucleosomes in chromatin regulatory mechanisms [3].

### TECHNICAL INFORMATION

<b>Storage</b>	Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid freeze/thaws.
<b>Formulation</b>	0.7 mg/mL mononucleosome in 35.7 µL 10 mM Tris HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol (13.6 µg protein, 25 µg DNA + protein).

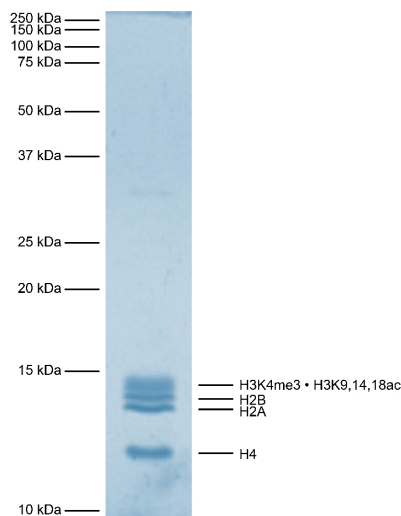
### APPLICATION NOTES

Heterotypic [H3K4me3 • H3K9,14,18ac] nucleosome 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. Trace amounts of TEV protease may be present (less than 5% of the preparation by densitometry, **Figure 1**). For a corresponding homotypic control, we recommend EpiCypher 16-0335.

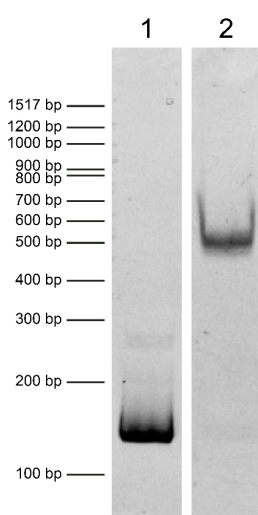
### GENE & PROTEIN INFORMATION

<b>UniProt ID</b>	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
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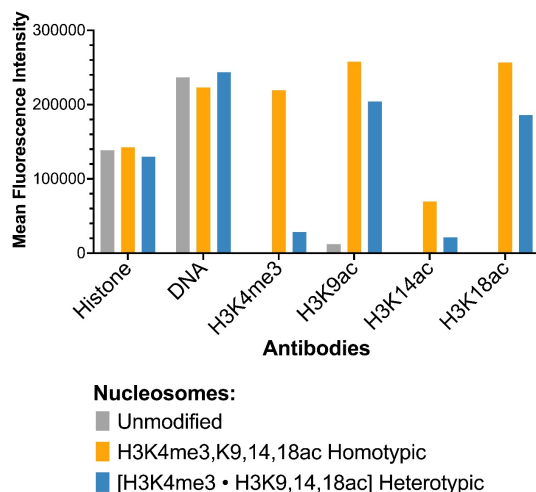
## VALIDATION DATA



**FIGURE 1 Protein gel data.** Coomassie stained SDS-PAGE gel of proteins in heterotypic [H3K4me3 • H3K9,14,18ac] nucleosome (1  $\mu$ g) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2B, H3K4me3 • H3K9,14,18ac, and H4) are indicated.



**FIGURE 2 DNA gel data.** Heterotypic [H3K4me3 • H3K9,14,18ac] 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; 75 ng). Free DNA is over 95% pure by densitometry. **Lane 2:** Intact heterotypic [H3K4me3 • H3K9,14,18ac] nucleosomes (400 ng).



**FIGURE 3 Luminex multiplexed specificity profiling.** Heterotypic [H3K4me3 • H3K9,14,18ac] nucleosome was assessed using a Luminex® based approach. The panel comprises biotinylated designer nucleosomes individually coupled to color-coded Luminex MagPlex® beads. Histone antibody (EMD Millipore MAB3422), DNA antibody (EMD Millipore MAB030), H3K4me3 antibody (EpiCypher 13-0060), H3K9ac antibody (RevMAb 31-1054-00), H3K14ac antibody (ThermoFisher MA5-32814), and H3K18ac antibody (Cell Signaling Technology 13998) were added (x-axis), and a second layer of anti-IgG\*Phycoerythrin (PE; BioLegend 406421 for anti-rabbit IgG or BioLegend 405307 for anti-mouse IgG) was used for detection. Data were generated using a Luminex FlexMAP 3D®. Histone and DNA antibodies bind nucleosomes in the panel equivalently, as expected. H3K4me3, H3K9ac, H3K14ac, and H3K18ac antibodies bind heterotypic [H3K4me3 • H3K9,14,18ac] nucleosomes at reduced strength in comparison to homotypic H3K4me3, K9, 14, 18ac nucleosomes (EpiCypher 16-0335).

## REFERENCES

- [1] Valsakumar & Voigt *Biochem. Soc. Trans.* (2024). PMID: 38778762
- [2] Lowary & Widom *J. Mol. Biol.* (1998). PMID: 9514715
- [3] Jain et al. *eLife* (2023). PMID: 37204295