

Heterotypic [H3 • H3K4me3] Recombinant Nucleosome, Biotinylated

Catalog No	16-0408	Species	Human
Lot No	24219001-81	Source	<i>E. coli</i> & synthetic DNA
Pack Size	25 µg	Tag	Biotinylated
Concentration	5.4 µM	MW	199,689 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 [H3 • H3K4me3] Recombinant Nucleosome, Biotinylated is a fully defined semi-synthetic nucleosome containing one unmodified histone H3 and one histone H3 with trimethylated lysine at position four. [H3 • H3K4me3] 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. Heterotypic [H3 • H3K4me3] nucleosomes fail to recruit known H3K4me3 binding proteins, including members of the TFIID, SETD1, and SIN3A/B complexes; chromatin remodelers NURF, CHD1, and CHD8; and PHD finger protein PHF2, suggesting that heterotypic nucleosomes may regulate transcription by affecting the recruitment of various chromatin regulatory complexes [3].

TECHNICAL INFORMATION

Storage	Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid freeze/thaws.
Formulation	1.08 mg/mL mononucleosome in 23.1 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 [H3 • H3K4me3] 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 16-0316.

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
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VALIDATION DATA

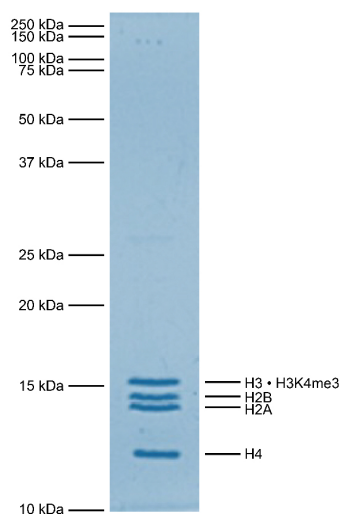


FIGURE 1 Protein gel data. Coomassie stained SDS-PAGE gel of proteins in heterotypic [H3 • H3K4me3] nucleosome (1 μ g) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2B, H3 • H3K4me3, and H4) are indicated.

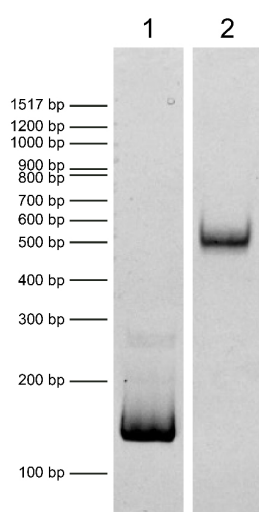


FIGURE 2 DNA gel data. Heterotypic [H3 • H3K4me3] nucleosome resolved via native PAGE and stained with ethidium bromide to visualize DNA. Both lanes are from the same gel. **Lane 1:** Free DNA (EpiCypher 18-0005; 75 ng). Free DNA is over 95% pure by densitometry. **Lane 2:** Intact heterotypic [H3 • H3K4me3] nucleosomes (400 ng).

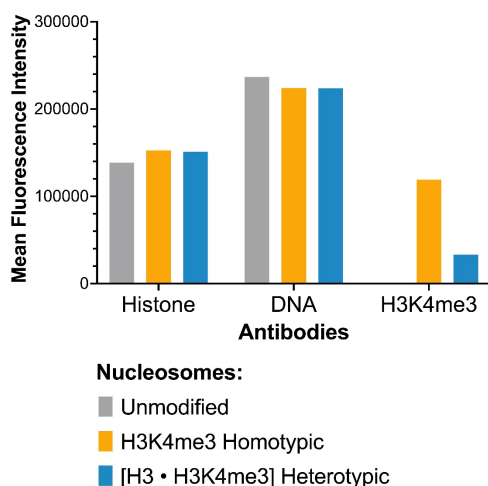


FIGURE 3 Luminex multiplexed specificity profiling. Heterotypic [H3 • H3K4me3] nucleosome was assessed using a Luminex® based approach. The panel comprises biotinylated designer nucleosomes individually coupled to color-coded Luminex MagPlex® beads. Histone antibody (EMD Millipore MAB3422), DNA antibody (EMD Millipore MAB030), and H3K4me3 antibody (EpiCypher 13-0060) 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 antibody binds heterotypic [H3 • H3K4me3] nucleosomes at reduced strength in comparison to homotypic H3K4me3 nucleosomes (EpiCypher 16-0316).

REFERENCES

- [1] Valsakumar & Voigt *Biochem. Soc. Trans.* (2024). PMID: 38778762
- [2] Lowary & Widom *J. Mol. Biol.* (1998). PMID: 9514715
- [3] Bryan et al. *bioRxiv* (2021). DOI: 10.1101/2021.02.08.430127