

Mononucleosomes (H2AX), Human Recombinant Biotinylated

Catalog No	16-0013	Species	Human
Lot No	17032001	Source	<i>E. coli</i> & synthetic DNA
Pack Size	50 µg	Tag	Biotinylated
Concentration	4.94 µM	MW	202,308 Da

DESCRIPTION

Mononucleosomes assembled from recombinant human histones expressed in *E. coli* (two each of histones H2AX, H2B, H3, and H4) wrapped by 147 base pairs of 601 positioning sequence DNA with a 5' biotin-TEG group. The nucleosome is the basic subunit of chromatin. H2AX is a variant of histone H2A that is phosphorylated on serine 139 by the ATM and ATR kinases in response to DNA double strand breakage. Phosphorylation of H2AX serves to recruit a number of DNA damage repair proteins, such as BRCA1 and NBS1.

TECHNICAL INFORMATION

Storage	Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid freeze/thaws.
Formulation	1.0 mg/mL purified recombinant mononucleosomes (27.4 µg protein by mass, 50 µg protein + DNA) in 50.0 µL 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 25 mM NaCl, 2 mM DTT, 20% glycerol.

APPLICATION NOTES

Mononucleosomes (H2AX), Human Recombinant Biotinylated are highly purified and are suitable for use as substrates in enzyme screening assays or for nucleosome binding experiments. The absence of post-translational histone modifications makes them ideal for conducting enzyme activity and screening assays. EpiCypher Mononucleosomes (H2AX), Human Recombinant Biotinylated do not contain free DNA which could alter assayed activities.

GENE & PROTEIN INFORMATION

UniProt ID	H2AX - P16104 (alt. names: H2a/x, Histone H2A.X) H2B - O60814 (alt. names: H2B K, HIRA-interacting protein 1) H3.1 - P68431 (alt. names: H3, H3/a, H3/b, H3/c, H3/d) H4 - P62805
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REFERENCES

- Lowary & Widom *J. Mol. Biol.* (1998). PMID: 9514715
Luger et. al. (1999) *Methods Mol. Biol.* 119:1-16. PMID: 10804500

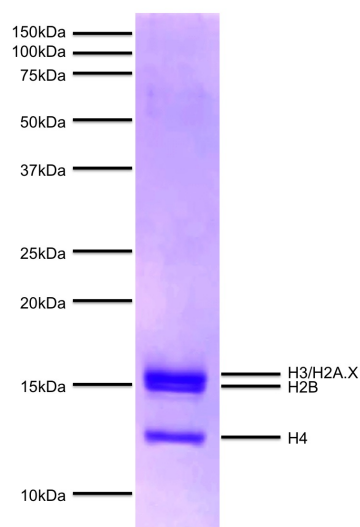


FIGURE 1 Protein gel data. Coomassie stained PAGE gel of proteins in Nucleosome (H2AX), Human Recombinant Biotinylated (1 µg) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2AX, H2B, H3 and H4) are indicated.

N.B. Histone H2AX co-migrates with histone H3.

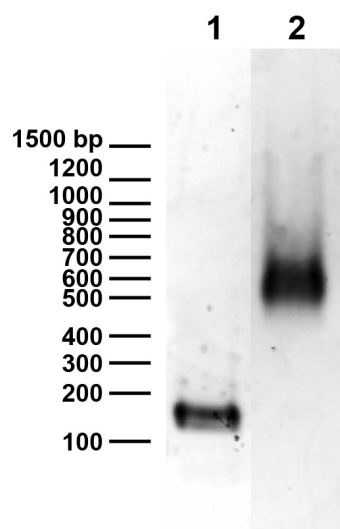


FIGURE 2 DNA gel data. Nucleosome (H2AX), Human Recombinant Biotinylated run on an agarose gel and stained with ethidium bromide to visualize DNA.

Lane 1: DNA extracted from nucleosomes (100 ng). **Lane 2:** Intact nucleosomes (400 ng).

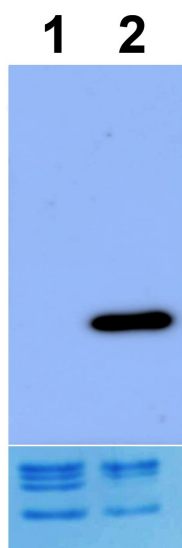


FIGURE 3 Western blot data. Western analysis of Nucleosome (H2AX), Human Recombinant Biotinylated. **Top Panel:** H2A-containing (Lane 1) and H2AX-containing nucleosomes (Lane 2) were probed with an anti-H2AX antibody and analyzed via ECL readout. Only the H2AX samples produced a detectable signal. **Bottom Panel:** Detail from Coomassie stained gel showing histones from H2A (Lane 1) and H2AX nucleosomes (Lane 2).