Mononucleosomes, Recombinant Human, Desthiobiotinylated

Catalog No. 16-0024
Lot No. 19231001
Pack Size 50 µg

Product Description:

Mononucleosomes assembled from recombinant human histones expressed in *E. coli* (two each of histones H2A, H2B, H3.1, and H4 Accession numbers: H2A-P04908; H2B-O60814; H3.1-P68431; H4-P62805) wrapped by 147 base pairs of 601 positioning sequence DNA. The nucleosome is the basic subunit of chromatin. The 601 sequence, identified by Lowary and Widom, is a 147-base pair sequence that has high affinity for histone octamers and is useful for nucleosome assembly and contains a 5' desthiobiotin-TEG group. Desthiobiotin has a weaker affinity ($K_d = 10^{-11}$) for avidin-family proteins than does biotin ($K_d = 10^{-15}$).

Formulation:

Purified recombinant mononucleosomes (50 µg total mass, 27.3 µg protein + 23.7 µg DNA in 42.5 µL) in 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 25 mM NaCl, 2 mM DTT, & 20% glycerol. Concentration of nucleosomes is 5.89 µM. Nucleosome molecular weight = 199,827.86 Da.

Storage and Stability:

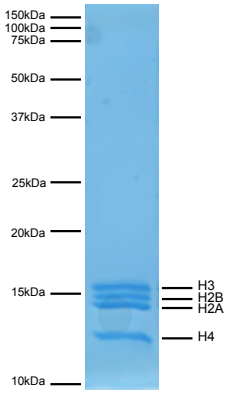
Stable for six (6) months at -80°C from date of receipt. For best results, aliquot and avoid multiple freeze/thaws.

Application Notes:

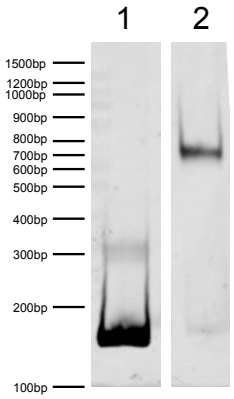
Mononucleosomes, Human Recombinant Desthiobiotinylated are highly purified and suitable for use as substrates in enzyme screening assays, structural studies, or effector protein binding experiments. The weaker affinity of desthiobiotin for avidin-family proteins allows nucleosomes to be eluted via addition of free biotin. The ability for these nucleosomes to be recovered intact is useful in many aspects of chromatin biology.

References:

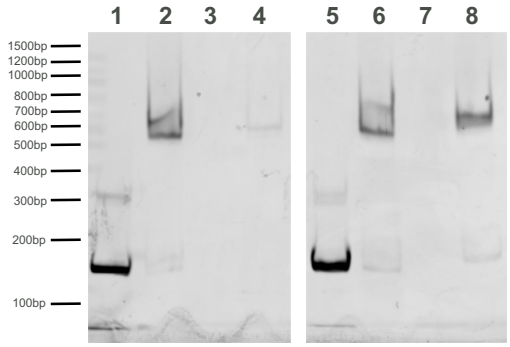
Lowary PT and J Widom (1998). *J Mol Biol* 276: 19-42.
Luger K et al (1999). *Methods Mol Biol* 119: 1-16.



Protein Gel Data: Coomassie stained SDS-PAGE of proteins in Mononucleosomes, Human Recombinant, Desthiobiotinylated (1 µg) demonstrates the purity of the histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2B, H3 and H4) are indicated.



DNA Gel Data: Mononucleosomes, Recombinant Human Desthiobiotinylated resolved via native PAGE and stained with ethidium bromide to visualize DNA. Lane 1: Free DNA (200 ng). Lane 2: Intact nucleosomes (400 ng).



Desthiobiotin Elution Data: Nucleosomes, Recombinant Human, Desthiobiotinylated efficiently elute from streptavadin magentic beads with the addition of 5mM free biotin. Nucleosomes, Recombinant Human, Biotinylated (EpiCypher Cat. No. 16-0006) do not elute from streptavadin magnetic beads under the same conditions. **Lane 1** EpiCypher Cat. No. 18-0005 DNA loading control (100ng) **Lane 2** EpiCypher Cat. No. 16-0006 input (200ng). **Lane 3** 1xTBST wash of beads. **Lane 4**. 5mM D(+)-biotin eluate. **Lane 5** EpiCypher Cat. No. 18-0024 DNA loading control (100ng) **Lane 6** EpiCypher Cat. No. 16-0024 input (200ng). **Lane 7** 1xTBST wash of beads. **Lane 8**. 5mM D(+)-biotin eluate. Nucleosomes remain intact throughout the elution process.