

Recombinant Mononucleosomes H3K27me3 (MLA) - biotin

Catalog No: 81135, 81835

Lot No: 08518001

Expressed In: *E. coli*

Quantity: 20, 1000 µg

Concentration: 0.54 µg/µl

Source: Human

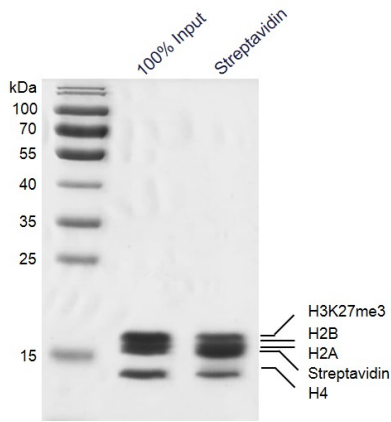
Buffer Contents: Recombinant Mononucleosomes H3K27me3 (MLA) - biotin (20 µg protein + 20 µg DNA) are supplied in 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 2 mM DTT, and 20% glycerol.

Background: Histone H3 is one of the core components of the nucleosome. The nucleosome is the smallest subunit of chromatin and consists of 146 base pairs of DNA wrapped around an octamer of core histone proteins (two each of H2A, H2B, H3 and H4). Histone H1 is a linker protein, present at the interface between the nucleosome core and DNA entry/exit points. Recombinant Histone H3K27me3 (MLA) has been generated using the patented Methylated Lysine Analog (MLA) technology. In MLA, methylated histones are generated via a chemical alkylation reaction that substitutes a methylated analog of lysine, aminoethylcysteine, for the existing lysine at the desired residue. Aminoethylcysteine is structurally and chemically similar to lysine, though it contains a sulfide substitution in place of the lysine γ -methylene. The MLA technique provides precise control over the site and degree of methylation. The MLA technology is covered under U.S. Patent No. 8,278,112.

Protein Details: Recombinant Mononucleosomes H3K27me3 (MLA) - biotin consist of a 167 bp of 601 DNA with a 5'-biotin tag and two molecules each of histones H2A that includes amino acids 1-130 (end) (accession number NM_003512), H2B that includes amino acids 1-126 (end) (accession number NM_003518), H3 trimethyl Lys27 (H3K27me3) (MLA) that includes amino acids 1-136 (end) (accession number NP_003520.1) with two substitutions (cysteine to serine at amino acid 96 and cysteine to alanine at amino acid 110), and H4 that includes amino acids 1-103 (end) (accession number NM_003548). All of these histones were expressed in *E. coli* cells. The molecular weight of histone octamer is 108 kDa.

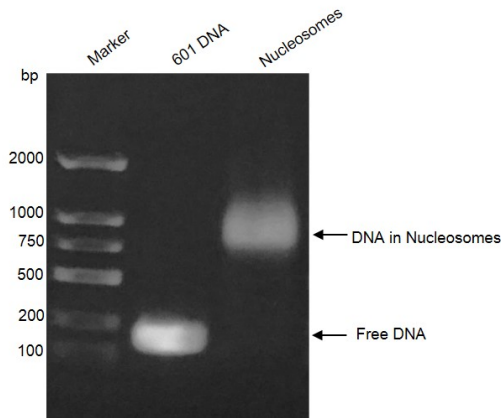
Application Notes: Recombinant histones are suitable for use as positive controls in the analysis of histone posttranslational modifications, as substrates for histone modification enzymes, or to generate chromatin *in vitro*.

Storage and Guarantee: Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is for research use only and is not for use in diagnostic procedures. This product is guaranteed for 6 months from date of arrival.



Streptavidin pull-down for Recombinant Mononucleosomes H3K27me3 (MLA) – biotin

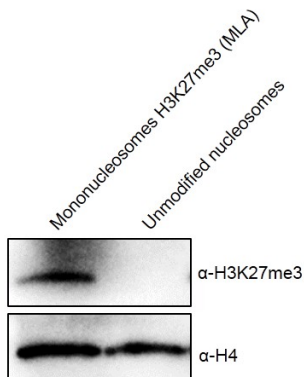
Recombinant Mononucleosomes were pulled down by streptavidin beads. Input nucleosomes (Lane 2) and the nucleosomes (Lane 3) pulled down by streptavidin were run on a 12.5% SDS-PAGE gel and stained with Coomassie blue. The SDS-PAGE gel result shows that most of biotinylated mononucleosomes H3K27me3 (MLA) are pulled down by streptavidin beads.



Recombinant Mononucleosomes H3K27me3 (MLA) - biotin agarose gel

Recombinant Mononucleosomes were run on a 2% agarose gel and stained with ethidium bromide. Lane 1: DNA marker. Lane 2: 601 DNA. Lane 3: Intact mononucleosomes H3K27me3.

Intact mononucleosomes H3K27me3 migrated much higher than free 601 DNA. The agarose gel shows that almost all of 601 DNA wrap histone octamers to form nucleosomes.



Western Blot analysis for Recombinant Mononucleosomes H3K27me3 (MLA) - biotin

Recombinant Mononucleosomes (Lane 1) and unmodified nucleosomes (Lane 2) were detected with H3K27me3 antibody and H4 antibody, respectively. H4 was detected as loading control. Only Recombinant Mononucleosomes H3K27me3 (MLA) - biotin can be detected by anti-H3K27me3 antibody.