

Recombinant Mononucleosomes H3K4me3/H3K27me3 - biotin

Catalog No: 81005, 81705

Expressed In: *E. coli* / Synthetic

Quantity: 10 µg

Concentration: 0.16 µg/µl

Source: Human

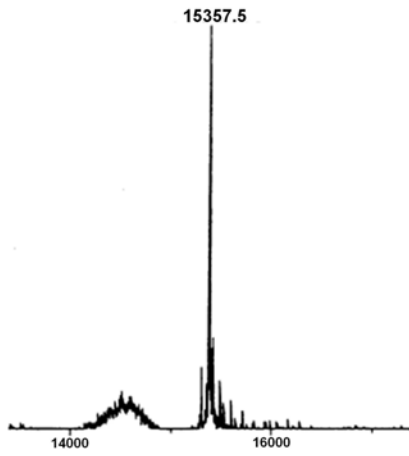
Buffer Contents: Recombinant Mononucleosomes H3K4me3/H3K27me3 - biotinylated (10 µg protein + 10 µg DNA) is supplied at a protein concentration of 0.16 µg/µl in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2 mM DTT and 20% glycerol.

Background: *In vivo*, histones are wrapped around by DNA in chromatin. Therefore, nucleosomes are more physiologically relevant substrates than histones and histone-derived peptides for *in vitro* studies. More importantly, some histone methyltransferases are significantly more active, as well as specific, when using nucleosomal substrates in HMT assays, such as DOT1L and NSD family enzymes. Nucleosomes are also widely used in histone methyltransferase screening assays to identify small molecular inhibitors for drug discovery.

Protein Details: Recombinant Mononucleosomes H3K4me3/H3K27me3 - biotinylated consist of a 167 bp of 601 DNA with 5' biotin tag and two molecules each of histones H2A that includes amino acids 1-130 (end) (accession number NP_003503.1), H2B that includes amino acids 1-126 (end) (accession number NP_003509.1), H3.1 that includes amino acids 1-136 (end) (accession number NP_003520.1) with trimethylation at lysine 4 and lysine 27, and H4 that includes amino acids 1-103 (end) (accession number NP_003539.1). Histone H2A, H2B and H4 were expressed in *E. coli* cells. Histone H3K4me3/H3K27me3 was synthesized completely. H3K4me3/H3K27me3 corresponds to the native histone sequence and does not contain any amino acid substitutions or residue analogs. The molecular weight of histone octamer is ~108 kDa.

Application Notes: Recombinant Mononucleosomes H3K4me3/H3K27me3 - biotinylated are suitable for use as substrate of enzymatic assay or other biochemical assay.

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.

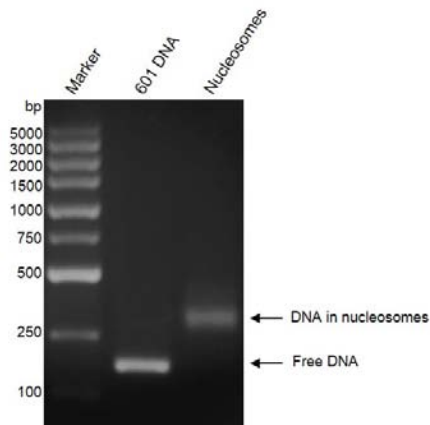


Mass Spec analysis of Recombinant Mononucleosomes H3K4me3/H3K27me3 - biotin.

Synthetic H3K4me3/H3K27me3 protein was analyzed by ESI-TOF mass spectrometry.

Expected mass = 15357.8 Da.

Determined mass = 15357.5 Da.



Recombinant Mononucleosomes H3K4me3/H3K27me3 - biotin, DNA gel.

Recombinant Mononucleosomes H3K4me3/H3K27me3 - biotin were run on a 2% agarose gel and stained with ethidium bromide. Lane 1: DNA marker. Lane 2: 601 DNA. Lane 3: Intact mononucleosomes H3K4me3/H3K27me3. Intact mononucleosomes H3K4me3/H3K27me3 migrated much higher than free 601 DNA.

The agarose gel result shows almost all of 601 DNA wrap histone octamers to form nucleosomes.



Western Blot analysis of Recombinant Mononucleosomes H3K4me3/H3K27me3 - biotin.

Unmodified nucleosomes (Lane 1) and Recombinant Mononucleosomes H3K4me3/H3K27me3 - biotin (Lane 2) were detected with antibody anti-H3K4me3, anti-H3K27me3 and anti-H4, respectively. H4 was detected as loading control.

Only Recombinant Mononucleosomes H3K4me3/H3K27me3 - biotin can be detected by anti-H3K4me3 and anti- H3K27me3 antibody.