Recombinant Mononucleosomes H3.1 (K4I)

Catalog No: 81257, 81957
Expressed In: *E. coli*

Quantity: 20, 1000 µg
Concentration: 0.8 µg/µl
Source: Human

Buffer Contents: Recombinant Mononucleosomes H3.1 (K4I) (20 µg protein + 20 µg DNA) is supplied in 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 2 mM DTT, and 20% glycerol.

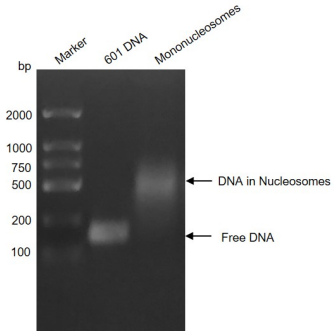
Background: *In vivo*, histones are wrapped around by DNA in chromatin. Therefore, nucleosomes are more physiologically relevant substrates than histones and histone-derived peptides for in vitro studies. More importantly, some histone methyltransferases are signifcantly more active, as well as specifc, 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. Histones are linked to tumorigenesis primarily through alterations in their PTMs and the enzymes regulating these modifications, suggesting that they might disrupt the reading, writing, and/or erasing of these marks. Mutations in histone H3 occur with high genetic penetrance within rare paediatric gliomas and sarcomas. In H3 variants, the mutation is most often a lysine-to-methionine (K-M) mutation, occasionally glycine mutations (G34R/V/W/L) occur too. According to researchers, mutations at H3K4: out of a total of 9 mutations at this site, 8 were a K4M/I substitution. More K-to-M/I mutations were observed, raising the possibility that the functional effects associated with known K-to-M/I changes (that is, function in a dominant fashion to block the methylation of corresponding lysines on wild type histones) may extend to additional contexts.

Protein Details: Recombinant Mononucleosomes H3.1 (K4I) consist of a 167 bp of 601 DNA 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), H3.1 that includes amino acids 1-136 (end) (accession number NM_003529) with a point mutation Lys4Ile, 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 Mononucleosomes H3.1 (K4I) is suitable for use as substrate for histone modification enzymes, or to generate chromatin *in vitro*

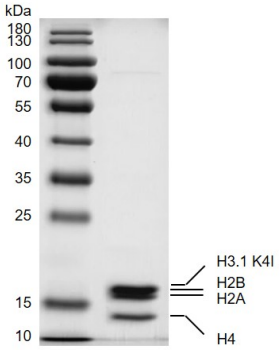
Storage and Guarantee: Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is guaranteed for 6 months from date of receipt.

This product is for research use only and is not for use in diagnostic procedures.



Recombinant Mononucleosomes H3.1 (K4I) DNA gel

Recombinant Mononucleosomes H3.1 (K4I) were run on a 2% agarose gel and stained with ethidium bromide. Lane 1: DNA marker. Lane 2: 601 DNA which was used for assembly of nucleosome. Lane 3: Intact mononucleosomes H3.1 (K4I). Intact Mononucleosomes H3.1 (K4I) migrated much higher than free 601 DNA. The agarose gel shows that almost all of 601 DNA wrapped histone octamers to form nucleosomes.

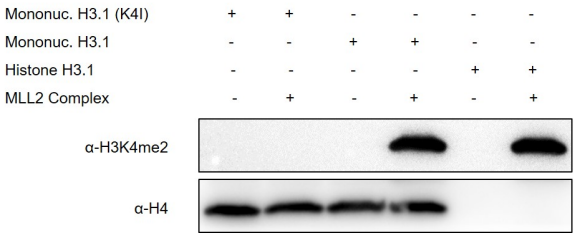


Recombinant Mononucleosomes H3.1 (K4I)

12.5% SDS-PAGE Coomassie staining

MW: 108 kDa

Purity: >95%



Western blot assay for Recombinant Mononucleosomes H3.1 (K4I)

2 µg Recombinant Mononucleosomes H3.1 (K4I) were incubated with 0 or 10 nM MLL2 Complex respectively in reaction buffer containing 50 mM Tris-HCl pH 8.6, 0.02% Triton X-100, 2 mM MgCl2, 1 mM TCEP and 50 µM SAM for 3 hr at room temperature. Half of the reactions were run on a 12.5% SDS-PAGE gel and detected with H3K4me2 antibody (Cat. No. 39679) and anti-H4 antibody (Cat. No. 39269), respectively. H4 was detected as loading control. Recombinant Mononucleosomes H3.1 (Cat# 81070) and Histone H3.1 (Cat. No. 31294) was used as a positive control. The result shows that no H3K4me2 products were generated when Mononucleosomes H3.1 (K4I) were incubated with MLL2 Complex.