Recombinant Mononucleosomes H3.3 (K18M) - biotin

Catalog No: 81288, 81988
Expressed In: *E. coli*

Quantity: 20, 1000 µg
Concentration: 0.82 µg/µl
Source: Human

Buffer Contents: Recombinant Mononucleosomes H3.3 (K18M) - biotin are supplied in 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 2 mM DTT, and 20% glycerol.

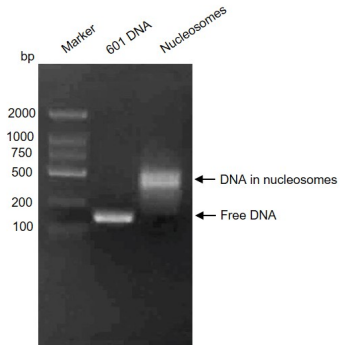
Background: *In vivo*, histones are wrapped around by DNA in chromatin. Therefore, nucleosomes are more physiologically relevant substrates than histones and histone-derived peptides for in vitro studies. More importantly, some histone methyltransferases are signifcantly more active, as well as specifc, 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. 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. Researchers found that mutations in the subset with a TMB ≤ 2 mutations per Mb included H3 (K27M) and H3 (G34W), and other mutations like H3 (E105K/Q), mutations at H3 N-terminal residues at or near PTM sites including R2, R8, K18 and R26, as well as residues in the acidic patch such as H2A residues E56, E64, E9, and E92 and H2B residues E105 and E133, which might act as oncohistones.

Protein Details: Recombinant Mononucleosomes H3.3 (K18M) - biotin consist of a 167 bp of 601 DNA without tags 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), H3.3 that includes amino acids 1-136 (end) (accession number NM_002107.6) with a point mutation Lys18Met, 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.

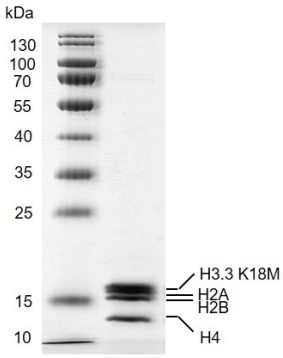
Application Notes: Recombinant Mononucleosomes H3.3 (K18M) - biotin are suitable for use as substrate for histone modification enzymes, or to generate chromatin *in vitro*.

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 arrival.



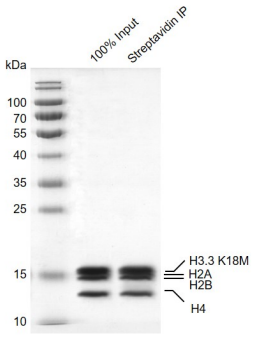
Recombinant Mononucleosomes H3.3 (K18M) - biotin DNA gel

Recombinant Mononucleosomes H3.3 (K18M) 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 nucleosomes. Lane 3: Intact mononucleosomes H3.3 (K18M). Intact mononucleosomes H3.3 (K18M) 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 H3.3 (K18M) - biotin protein gel

12.5% SDS-PAGE gel stained with Coomassie Blue
MW: 108 kDa
Purity: >95%



Streptavidin pull down assay for Recombinant Mononucleosomes H3.3 (K18M) - biotin

24 µg biotinylated mononucleosomes were incubated with 10 µl streptavidin beads for 1 hr at 4° C. Streptavidin beads were washed 3 times with 1 ml binding buffer. Then the beads were added 60 µl 2×SDS loading buffer and boiled for 10 min at 95°C. 2.4 µl samples were loaded and run on a 12.5% SDS-PAGE gel and stained by Coomassie blue. * indicates streptavidin. The SDS-PAGE gel result shows that almost all of biotinylated mononucleosomes were pulled down by streptavidin beads.