

Recombinant Polynucleosomes H3.3 (K36M)

Catalog No: 31563, 31963

Lot No: 27016001

Expressed In: *E. coli*

Quantity: 20, 1000 µg

Concentration: 0.8 µg/µl

Source: Human

Buffer Contents: Human Recombinant Polynucleosomes H3.3 (K36M) (20 µg protein+ 24 µg DNA) are supplied in 10 mM Tris pH 8.0, 1 mM EDTA, 2 mM DTT, 20% glycerol. Please refer to product insert upon arrival for lot-specific concentration.

Background: *In vivo*, the nucleosome is the basic structural unit of chromatin. It is comprised of about 146 bp of DNA wrapped around a core of eight histones of four different types: H2A, H2B, H3 and H4. Histones are subject to posttranslational modifications, such as methylation, acetylation, phosphorylation and mono-ubiquitination. Histone modifications influence multiple chromatin-templated processes such as gene transcription, DNA repair and recombination. Besides the “major” histones, there are some histone variants in specific regions of chromatin or in specific cell types. Histone variants are involved in multiple processes including chromosome segregation, DNA repair, transcriptional regulation and mRNA processing.

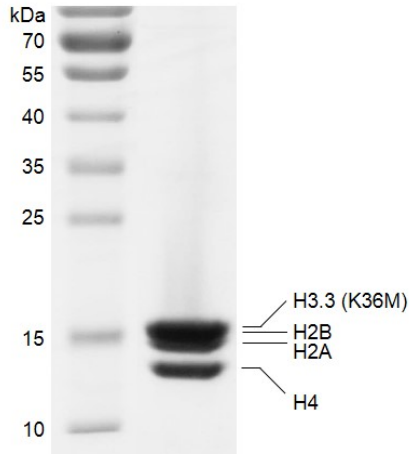
Histone H3.3 point mutations (K27 and G34) are found in 1/3 of pediatric glioblastomas. Up to 78% of diffuse intrinsic pontine gliomas (DIPGs) carry K27M and 36% of non-brainstem gliomas carry either K27M or G34R/V mutations. High-frequency mutation of histone H3 to K36M is observed in chondroblastomas, as is the mutation G34W/L in giant cell tumors of bone, diseases of adolescents and young adults. Histone H3.3 mutations drive pediatric glioblastoma through upregulation of MYCN.

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 Polynucleosomes H3.3 (K36M), Human, consist of 5000 bp of DNA (plasmid pG5E4) 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_005324) with a point mutation Lys36Met, and H4 that includes amino acids 1-103 (end) (accession number NM_003548). Plasmid pG5E4 contains 9 of 5S rDNA nucleosome positioning sequences of *L. variegatus*, 5 of GAL4 binding sites and E4 promoter. Every 5S rDNA can wrap one histone octamer to form a nucleosome. 5 of GAL4 binding sites and E4 promoter can wrap histone octamers to form dinucleosomes. A pG5E4 plasmid can warp histone octamers to form 11 nucleosomes. The recombinant protein is >95% pure by SDS-PAGE

Application Notes: Recombinant Polynucleosomes H3.3 (K36M) are suitable as substrate for use in the study of enzyme kinetics, inhibitor screening, and selectivity profiling.

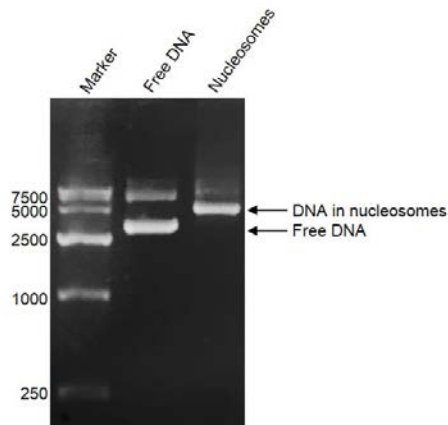
Storage and Guarantee: Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is for research use only and is not for use in diagnostic procedures. This product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



Recombinant Polynucleosomes H3.3 (K36M)

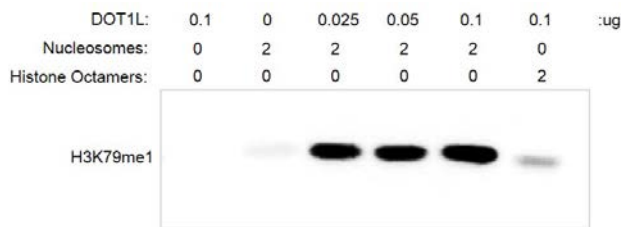
Polynucleosomes were run on a 12% SDS-PAGE gel and stained with Coomassie Blue.

The purity of the polynucleosomes H3.3 (K36M) is $\geq 95\%$.



DNA Gel-shift assay for Recombinant Polynucleosomes H3.3 (K36M).

Polynucleosomes H3.3 (K36M) and free plasmid DNA were run on a 1% agarose gel and stained with ethidium bromide. Intact polynucleosomes migrate much higher than free DNA, thus the DNA resolves at a higher molecular weight when nucleosome-bound.



Histone methyltransferase activity assay comparing recombinant polynucleosomes and histone octamers as substrates.

2 μg Recombinant Polynucleosomes H3.3 (K36M) were incubated with DOT1L (Cat# 31474) in reaction buffer for 3 hours at room temperature. Western Blot was used for detecting the generation of reaction products (H3K79me1, Cat# 39921). DOT1L only and polynucleosomes only were used as negative control.

The Western Blot result shows that polynucleosomes are more suitable substrate for DOT1L than histone octamers.