Recombinant Polynucleosomes (H3.3)

Catalog No: 31468, 31868
Expressed In: *E. coli*

Quantity: 20, 1000 µg
Concentration: 1.4 µg/µl
Source: Human

Buffer Contents: Human recombinant polynucleosomes (H3.3) (20 µg protein+ 24 µg DNA) are supplied at a protein concentration of 1.4 µg/µl in 10 mM Tris pH 8.0, 1 mM EDTA, 2 mM DTT, 20% glycerol.

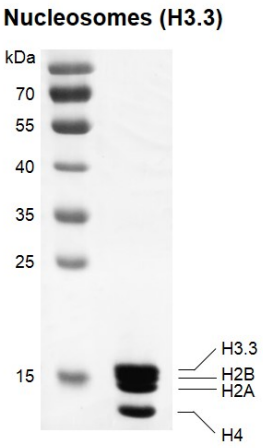
Background: *In vivo*, histones are wrapped around by DNA in chromatin. Therefore, nucleosomes are more physiologically relevant substrates than histones and histone-derived peptides for *in vitro* studies. More importantly, some histone methyltransferases are significantly more active, as well as specific, 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. Histone H3.1 and Histone H3.3 are the two main Histone H3 variants found in plants and animals. They are known to be important for gene regulation. Histone H3.1 and H3.3 have been shown to demonstrate unique genomic localization patterns thought to be associated with their specific functions in regulation of gene activity. Specifically, Histone H3.1 localization is found to coincide with genomic regions containing chromatin repressive marks (H3K9me3, H3K27me3 and DNA methylation), whereas Histone H3.3 primarily colocalizes with marks associated with gene activation (H3K4me3, H2BK120ub1, and RNA pol II occupancy). Deposition of the Histone H3.1 variant into the nucleosome correlates with the canonical DNA synthesis-dependent deposition pathway, whereas Histone H3.3 primarily serves as the replacement Histone H3 variant outside of S-phase, such as during gene transcription. Aberrant localization of these variants is also known to correlate with certain cancers.

Protein Details: Recombinant Polyucleosomes (H3.3), 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), 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.

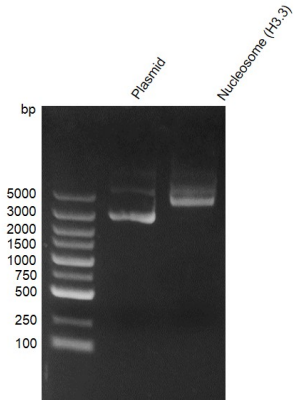
Application Notes: Recombinant Polynucleosomes (H3.3) are suitable for use in the study of enzyme kinetics, inhibitor screening, and selectivity profiling.

References:
This product was used in the following publications:
Cel Stem Cell (2019). 22(3):428-444. PMID: 29499155.
J. Cell Sci. (2016). 129(12): 2448-61. PMID: 27149922. (*in vitro* kinase 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.



Recombinant Polynucleosomes (H3.3) protein gel.
Recombinant Polynucleosomes (H3.3) were run on a 12% SDS-PAGE gel and stained with Coomassie Blue.



Recombinant Polynucleosomes (H3.3) protein DNA Gel-shift assay
Polynucleosomes (H3.3) and free plasmid DNA were run on 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 (HMT) Assay comparing Recombinant Polynucleosomes (H3.3) and histone octamers as substrates.

2 µg Recombinant Polynucleosomes (H3.3) were incubated with 0 µg , 0.25 µg, 0.5 µg and 1 µg NSD2-SET (Cat# 31476) in reaction buffer for 3 hours at room temperature, respectively. Western Blot was used to detect the generation of reaction products (H3K36me2, Cat# 39891). NSD2-SET only and polynucleosomes only were used as negative control. The Western Blot result shows that polynucleosomes are more suitable substrate for NSD2 than histone octamers.