# Recombinant Polynucleosomes (H3.1)

**Catalog No: 31466, 31866**
**Expressed In:** *E. coli*

**Quantity:** 20, 1000 µg
**Concentration:** 0.55 µg/µl
**Source:** Human

**Buffer Contents:** Recombinant Polynucleosomes (H3.1) (20 µg protein + 24 µg DNA) are supplied at a concentration of 0.55 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.

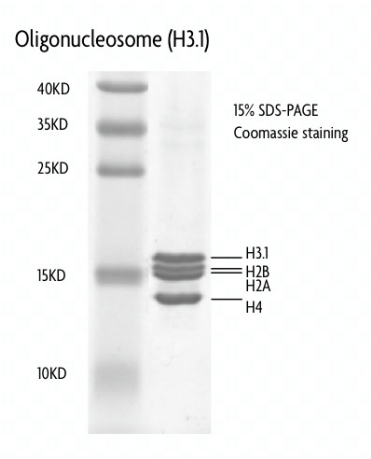
**Protein Details:** Recombinant Polynucleosomes (H3.1), Human consists of 5000 bp of DNA (plasmid pG5E4) and two molecules each of histones H2A that includes amino acids 1-130 (end) (accession number NM_003512), H2B that includes amino acids 1-126 (end) (accession number NM_003518), H3.1 that includes amino acids 1-136 (end) (accession number NM_003529), 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 (H3.1) are suitable for use in the study of enzyme kinetics, inhibitor screening, and selectivity profiling.

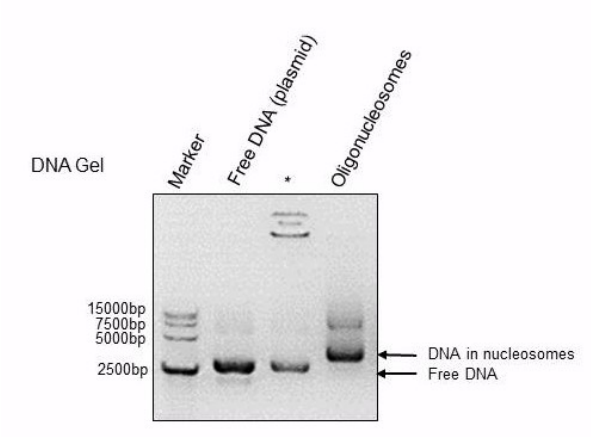
**HMT Assay Conditions:** 50 mM TrisCl, pH 8.6, 0.02% Triton X-100, 2 mM $MgCl_2$, 1 mM TCEP, 100 µM SAM, 30 ng/µl Recombinant Polynucleosomes (H3.1), 30 ng/µl DOT1L (1-416 aa) at 2 hours at room temperature. Activity was detected by fluorography.

**References:**
This product was used in the following publications:
*J. Cell Sci.* (2016). 129(12): 2448-61. PMID: 27149922.

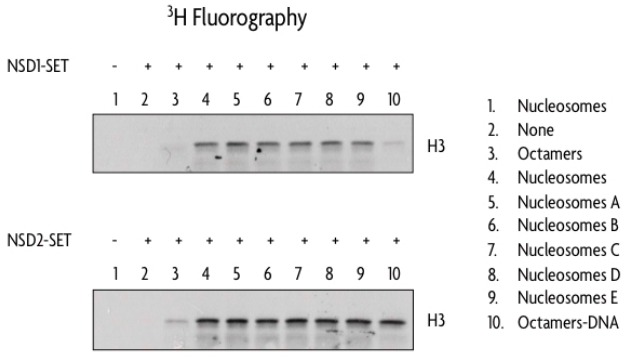
**Storage and Guarantee:** Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is for research use only and is not for use in diagnostic procedures. This product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




**Recombinant Polynucleosomes (H3.1) protein gel.**
Recombinant Polynucleosomes (H3.1) run on an SDS-PAGE gel and stained with Coomassie Blue.




**Recombinant Polynucleosomes (H3.1) protein DNA Gel-shift assay**
Polynucleosomes (H3.1) and free plasmid DNA were run on a 1% agarose gel and stained with ethidium bromide. Intact polynucleosomes migrate higher than free DNA, thus the DNA resolves at a higher molecular weight when nucleosome-bound. * represents the mixture of DNA and histone octamers (incorrect assembly).




**Recombinant Polynucleosomes (H3.1) protein activity assay.**
Results show that polynucleosomes are better substrates than octamers for NSD1-SET and NSD2-SET. Lanes 5-9 represent polynucleosomes that have been subjected to subsequent freeze-thaw cycles to demonstrate their stability. Activity was detected by fluorography.