Recombinant Mononucleosomes (H3.3)

Catalog No: 81071, 81771
Lot No: 30717001
Expressed In: *E. coli*

Quantity: 50, 1000 µg
Concentration: 0.75 µg/µl
Source: Human

Buffer Contents: Recombinant Mononucleosomes (H3.3) (50 µg protein + 50 µg DNA) are supplied at a protein concentration of 0.75 µg /µl in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2 mM DTT and 20% glycerol.

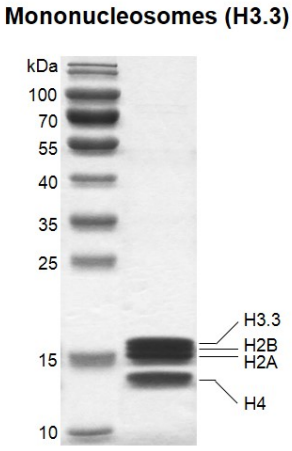
Background: *In vivo*, histones are wrapped around by DNA in chromatin. Therefore, nucleosomes are more physiologically relevant substrates than histones and histone-derived peptides for in vitro studies. More importantly, some histone methyltransferases are significantly more active, as well as specific, 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 Mononucleosomes (H3.3) consist of a 167 bp of 601 DNA without any tag 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). All of these histones were expressed in *E. coli* cells. The molecular weight of histone octamer is 108 kDa.

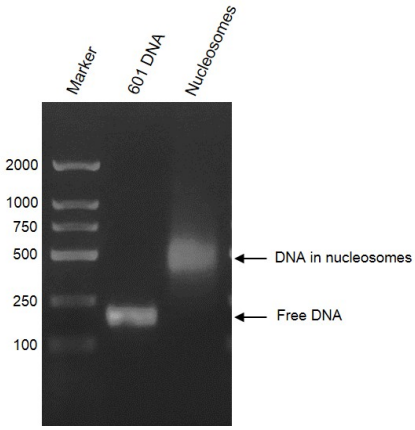
Application Notes: Recombinant Mononucleosomes (H3.3) are suitable for use in the study of enzyme kinetics, inhibitor screening, and selectivity profiling.

HMT Assay Conditions: 2 µg Recombinant Mononucleosomes (H3.3) were incubated with NSD2-SET (Cat. No. 31476) in 30 µl reaction system including 50 mM Tris-HCl pH 8.6, 0.02% Triton X-100, 2 mM MgCl2, 1 mM TCEP, 50 µM SAM for 3 hours at room temperature. Activity was detected by Western blot.

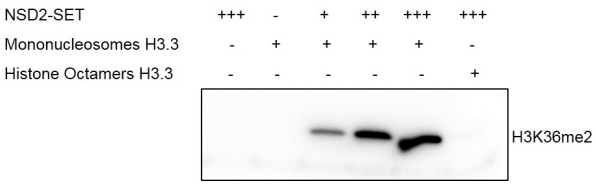
Storage and Guarantee: Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is for research use only and is not for use in diagnostic procedures. This product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



Recombinant Mononucleosomes (H3.3) SDS-PAGE gel
13% SDS-PAGE Coomassie staining
MW: 108 kDa
Purity: >95%



Recombinant Mononucleosomes (H3.3) DNA agarose gel
Mononucleosomes (H3.3) were run on an 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 nucleosome. Intact nucleosomes migrate much higher than free DNA.
The agarose gel result shows almost all of 601 DNA wrapp histone octamer to form nucleosomes.



Western Blot for activity detection of Mononucleosomes (H3.3)2 µg Recombinant Mononucleosomes (H3.3) were incubated with 0 µg, 0.25 µg, 0.5 ug, 1 µg NSD2-SET (Cat. No. 31476) in reaction buffer for 3 hours at room temperature, respectively. Western blot was used to detect the generation of reaction products (H3K36me2). Recombinant Histone Octamers were used as control substrates.
The Western Blot result showed that nucleosomes were more suitable substrate for NSD2-SET than histone octamers.