Recombinant Mononucleosomes (H3.1) - biotinylated

Catalog No: 31467, 31867
Expressed In: *E. coli*

Quantity: 20, 1000 µg
Concentration: 0.5 µg/µl
Source: Human

Buffer Contents: Recombinant Mononucleosomes (H3.1) - biotinylated (20 µg protein + 20 µg DNA) is supplied at a protein concentration of 0.5 µg/µl in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2 mM DTT and 20% glycerol.

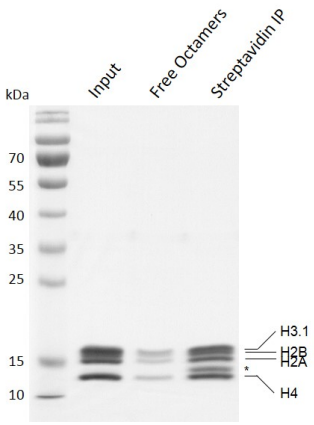
Background: *In vivo*, histones are wrapped around by DNA in chromatin. Therefore, nucleosomes are more physiologically relevant substrates than histones and histone-derived peptides for *in vitro* studies. More importantly, some histone methyltransferases are significantly more active, as well as specific, 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 (H3.1) - biotinylated consists of 167 bp of 601 DNA with 5' biotin 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), H3.1 that includes amino acids 1-136 (end) (accession number NM_003529), and H4 that includes amino acids 1-103 (end) (accession number NM_003548). All of these histones were expressed in E. coli cells. The molecular weight of histone octamer is 108 kDa. The recombinant protein is >95% pure by SDS-PAGE.

Application Notes: Recombinant Mononucleosomes (H3.1) - biotinylated is suitable for use in the study of enzyme kinetics, inhibitor screening, and selectivity profiling.

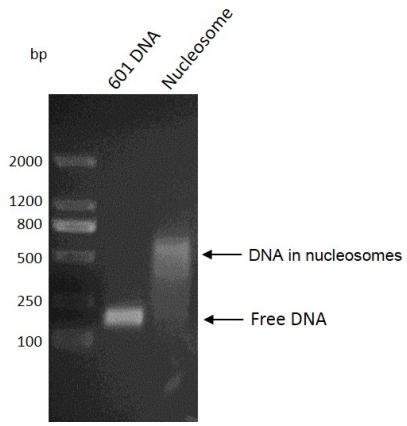
HMT Assay Conditions: 2 ug mononucleosomes were incubated with different concentrations of NSD2-SET (Cat# 31476) in reaction buffer including 50 mM TrisCl, pH 8.6, 0.02% Triton X-100, 2 mM MgCl2, 1 mM TCEP, 50 µM SAM for 3 hours at room temperature. Activity was detected by Western blot.

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.



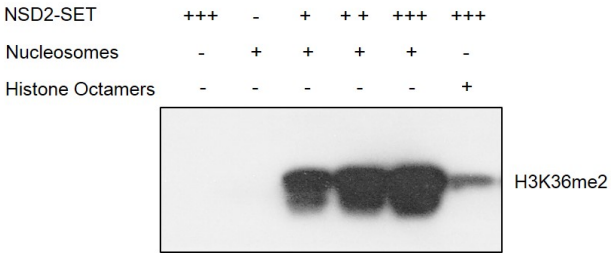
Recombinant Mononucleosomes (H3.1) - biotinylated protein gel.
40 µg mononucleosomes were incubated with 15 µl streptavidin beads for 1 h at 4C. Samples were centrifuged at 4C, and supernatant was labelled as free histone octamers. Samples were run on a 12.5% SDS-PAGE gel and stained by Commassie blue.

The SDS-PAGE gel result showed that more than 80% biotinylated mononucleosomes were pulled down by streptavidin beads. * indicates streptavidin.



Recombinant Mononucleosomes (H3.1) - biotinylated DNA gel.
Mononucleosomes were run on an agarose gel and stained with ethidium bromide. Lane 1: 601 DNA which was used for assembly of mononucleosomes. Lane 2: Intact mononucleosomes. Intact mononucleosomes migrate much higher than free DNA.

The agarose gel result showed almost all of the 601 DNA wrapped histone octamers to form mononucleosomes.



Recombinant Mononucleosomes (H3.1) - biotinylated activity.
2 µg mononucleosomes were incubated with 0 µg, 0.25 µg, 0.5 µg, 1 µg NSD2-SET (Cat# 31476) in reaction buffer for 3 h at room temperature, respectively. Western blot was used for detecting the generation of reaction products (H3K36me2). Recombinant Histone Octamers were used as a control substrate.

The Western blot result shows that mononucleosomes are a more suitable substrate for NSD2-SET than histone octamers.