Recombinant Mononucleosomes H3.3 (G34W) - biotin

Catalog No: 81011, 81711
Lot No: 18717001
Expressed In: *E. coli*

Quantity: 20, 1000 µg
Concentration: 0.8 µg/µl
Source: Human

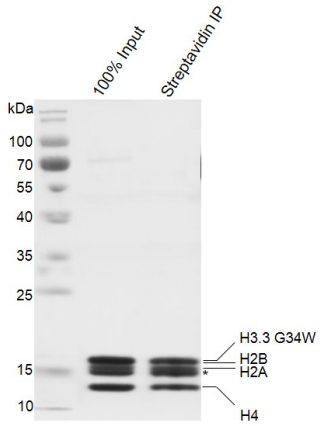
Buffer Contents: Recombinant Mononucleosomes H3.3 (G34W) - biotinylated (20 µg protein + 20 µg DNA) is supplied at a protein concentration of 0.8 µg/µl in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2 mM DTT and 20% glycerol.

Background: *In vivo,* the nucleosome is the basic structural unit of chromatin. It consists 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, mono-ubiquitination, tec. 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 were involved in multiple biology 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 mutations in histone H3 include K36M in chondroblastomas and 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 Mononucleosomes H3.3 (G34W) - biotinylated consist of a 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 NP_003503.1), H2B that includes amino acids 1-126 (end) (accession number NP_003509.1), H3.3 that includes amino acids 1-136 (end) (accession number NP_002098.1) with a point mutantion Gly34Trp, and H4 that includes amino acids 1-103 (end) (accession number NP_003539.1). All of these histones were expressed in *E. coli* cells. The molecular weight of the histone octamer is ~108 kDa. The purity of the recombinant protein is ≥95% by SDS-PAGE analysis.

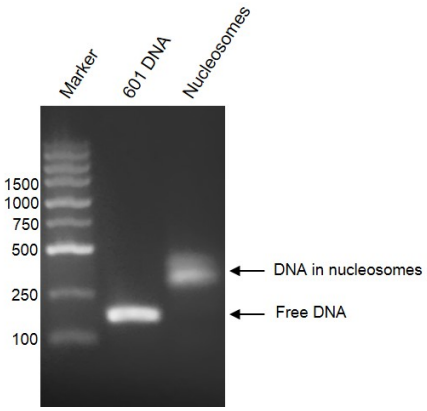
Application Notes: Recombinant Mononucleosomes H3.3 (G34W) - biotinylated are suitable for use in the study of enzyme kinetics, inhibitor screening, and selectivity profiling.

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.

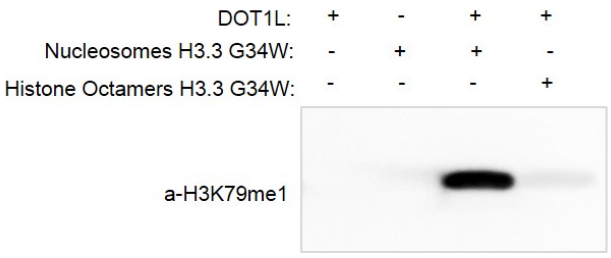


Streptavidin pull-down for Recombinant Mononucleosomes H3.3 (G34W) – biotin.
Recombinant Mononucleosomes H3.3 (G34W) - biotinylated were pulled down by streptavidin beads. Input nucleosomes (Lane 2) and the nucleosomes (Lane 3) pulled down by streptavidin were run on a 12.5% SDS-PAGE gel and stained with Coomassie blue.

The SDS-PAGE gel result shows that almost all of biotinylated mononucleosomes H3.3 (G34W) are pulled down by streptavidin beads. * indicates streptavidin.



Recombinant Mononucleosomes H3.3 (G34W) – biotin, DNA gel.
Recombinant Mononucleosomes H3.3 (G34W) - biotinylated were run on a 2% agarose gel and stained with ethidium bromide. Lane 1: DNA marker. Lane 2: 601 DNA. Lane 3: Intact monnucleosomes H3.3 (G34W). Intact mononucleosomes H3.3 (G34W) migrated much higher than free 601 DNA.
The agarose gel result shows almost all of 601 DNA wrap histone octamers to form nucleosomes.



Histone methyltransferase activity assay comparing recombinant nucleosomes and histone octamers as substrates.
2 µg biotinylated mononucleosomes H3.3 (G34W) were incubated with 0.2 µg DOT1L (1-420) in 30 µl reaction system containing 50 mM Tris-HCl, pH 8.6, 0.02% Triton X-100, 2 mM MgCl2, 1 mM TCEP, 50 µM SAM for 3 h at room temperature. 6 µl reaction products were loaded and run on a 12.5% SDS-PAGE gel. Western Blot was used to detect the generation of reaction products (Anit-H3K79me1, Cat. No. 39921). DOT1L only was used as negative control.