Recombinant Mononucleosomes (H1.2) - biotin

Catalog No: 81273, 81973
Expressed In: *E. coli*

Quantity: 20, 1000 µg
Concentration: 0.9 µg/µl
Source: Human

Buffer Contents: Recombinant Mononucleosomes (H1.2) - biotinylated (20 µg protein + 20 µg DNA) are in 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 2 mM DTT, 20% glycerol.

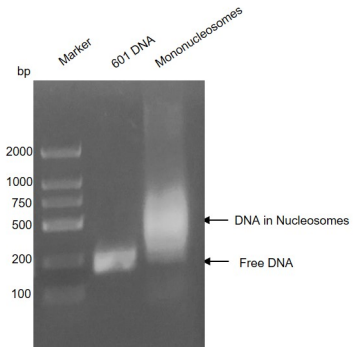
Background: Histones are basic nuclear proteins responsible for nucleosome structure of the chromosomal fiber in eukaryotes. Two molecules of each of the four core histones (H2A, H2B, H3, and H4) form an octamer, around which approximately 146 bp of DNA is wrapped in repeating units, called nucleosomes. In vivo, histones are wrapped around by DNA in chromatin. Therefore, nucleosomes are more physiologically relevant substrates than histones and histonederived 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. The called linker histone, H1, binds to linker DNA between nucleosomes forming the macromolecular structure known as the chromatin fiber. So it is necessary for the condensation of nucleosome chains into higher-order structured fibers. It can also act as a regulator of individual gene transcription through chromatin remodeling, nucleosome spacing and DNA methylation. Histone H1.2 is a replication-dependent histone that is a member of the histone H1 family.

Protein Details: Recombinant Mononucleosomes (H1.2) - biotinylated consist of a 204 bp of 601 DNA with 5' biotin tag (48 bp and 10 bp on 5' and 3' of 146 bp core DNA, respectively) 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), and histone H1.2 that includes amino acids 1-213 (end) (accession number NP_005310.1). All of these histones were expressed in E. coli cells. The molecular weight of histone octamer and histone H1.2 are 108 kDa and 21.4 kDa, respectively.

Application Notes: Recombinant Mononucleosomes (H1.2) - biotinylated are suitable for use as substrate of enzymatic assay or other biochemical assay.

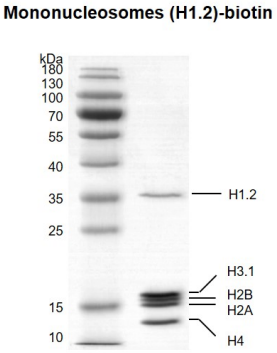
Storage and Guarantee: Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is guaranteed for 6 months from date of receipt.

This product is for research use only and is not for use in diagnostic procedures.



Recombinant Mononucleosomes (H1.2) - biotin DNA gel

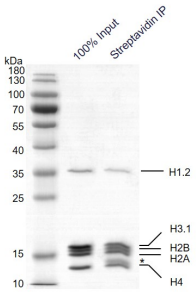
Recombinant Mononucleosomes (H1.2) - biotinylated were run on a 2% agarose gel and stained with ethidium bromide. Lane 1: DNA marker. Lane 2: 601 DNA. Lane 3: Intact monnucleosomes (H1.2) - biontinylated. Intact mononucleosomes (H1.2) - biotinylated migrated much higher than free 601 DNA. The agarose gel result shows almost all of 601 DNA wrap histone octamers to form nucleosomes.



Recombinant Mononucleosomes (H1.2) - biotin

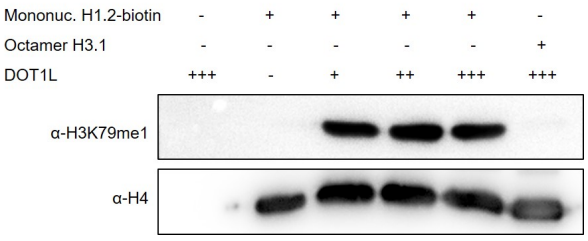
13% SDS-PAGE gel with Coomassie Blue staining

Purity: ≥95%



Streptavidin pull down assay for Recombinant Mononucleosomes (H1.2) - biotin

24 µg biotinylated mononucleosomes were incubated with 10 µl streptavidin beads for 1 hr at 4° C. Then beads were added to 60 µl 2×SDS loading buffer and boiled for 10 min at 95°C. 2.5 µl samples were loaded and run on a 12.5% SDS-PAGE gel and stained by Coomassie Blue. * indicates streptavidin. The SDS-PAGE gel result showed that about 60% of biotinylated mononucleosomes were pulled down by streptavidin beads.



Western blot assay for Recombinant Mononucleosomes (H1.2) - biotin

2 µg Recombinant Mononucleosomes were incubated with 0, 12.5, 25, 50 ng DOT1L (Cat. No. 31474) respectively in reaction buffer containing 50 mM Tris-HCl pH 8.6, 0.02% Triton X-100, 2 mM MgCl2, 1 mM TCEP and 50 µM SAM for 2 hr at room temperature. Half of the reactions were run on a 12.5% SDS-PAGE gel and detected with H3K79me1 antibody (Cat.No. 39921) and anti-H4 antibody (Cat. No. 39269), respectively. DOT1L protein and Recombinant Histone Octamers were used as controls. H4 was detected as the loading control. The result shows that mononucleosomes are better than octamers as substrates for DOT1L.