

Recombinant Mononucleosomes (H1.2)

Catalog No: 81272, 81972

Expressed In: *E. coli*

Quantity: 20, 1000 µg

Concentration: 0.9 µg/µl

Source: Human

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

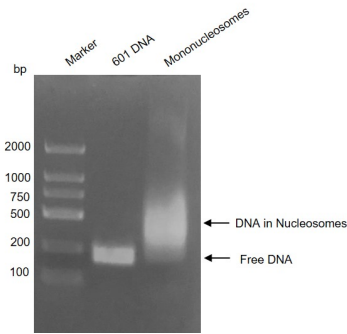
Background: Histones are basic nuclear proteins responsible for nucleosome structure of the chromosomal fiber in eukaryotes. Two molecules of each of the four core histones (H2A, H2B, H3, and H4) form an octamer, around which approximately 146 bp of DNA is wrapped in repeating units, called nucleosomes. In vivo, histones are wrapped around by DNA in chromatin. Therefore, nucleosomes are more physiologically relevant substrates than histones and 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. 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) consist of a 204 bp of 601 DNA (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) 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.

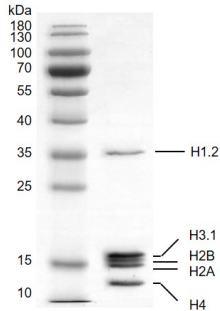
This product is for research use only and is not for use in diagnostic procedures.



Recombinant Mononucleosomes (H1.2) DNA gel

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

Mononucleosomes (H1.2)



Recombinant Mononucleosomes (H1.2) protein gel

13% SDS-PAGE with Coomassie Blue staining
Purity: $\geq 95\%$

Western blot assay for Recombinant Mononucleosomes (H1.2)

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 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.

