

Recombinant Mononucleosomes (H2A.Bbd) - biotinylated

Catalog No: 31556, 31956**Expressed In:** *E. coli***Quantity:** 20, 1000 µg**Concentration:** 1.2 µg/µl**Source:** Human

Buffer Contents: Recombinant Mononucleosomes (H2A.Bbd) - biotinylated (20 µg protein + 20 µg DNA) are supplied at a protein concentration of 1.2 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 is comprised of about 146 bp of DNA wrapped around a core of eight histones, made up of four different types: H2A, H2B, H3 and H4. Histones are subject to posttranslational modifications, such as methylation, acetylation, phosphorylation, and mono-ubiquitination. 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 are involved in multiple processes, including chromosome segregation, DNA repair, transcriptional regulation and mRNA processing.

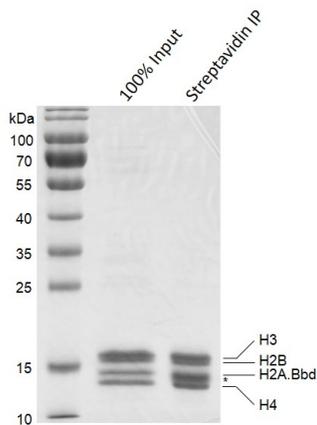
H2A.Bbd (Barr Body Deficient) is a histone H2A family member. H2A.Bbd is one of the most rapidly evolving variants, which shares 48% sequence identity with the major H2A histone. H2A.Bbd is excluded from the inactive X chromosome and is enriched in active regions of genome. H2A.Bbd is enriched at sites of DNA synthesis. H2A.Bbd nucleosomes are less stable than those bearing the major H2A histone and to organize only 118 bp of DNA, indicating that DNA in H2A.Bbd chromatin may be more accessible and, thus, more amenable to transcription. H2A.Bbd is involved in mammalian spermiogenesis.

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 (H2A.Bbd) - biotinylated, consist of 167 bp of 601 DNA with a 5' biotin tag and two molecules each of histones H2A.Bbd that includes amino acids 1-115 (end) (accession number NP_001017991.1), H2B that includes amino acids 1-126 (end) (accession number NP_003509.1), H3 that includes amino acids 1-136 (end) (accession number NP_003520.1), and H4 that includes amino acids 1-103 (end) (accession number NP_003539.1). All of these histones were expressed in *E. coli*. The molecular weight of the histone octamer is ~107 kDa. The recombinant protein is >95% pure by SDS-PAGE.

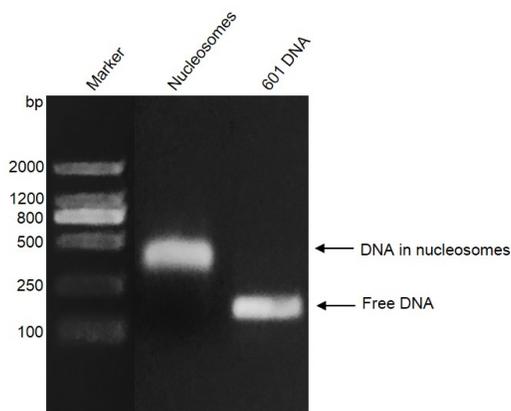
Application Notes: Recombinant Mononucleosomes (H2A.Bbd) - biotinylated, are suitable for use as substrates in the study of enzyme kinetics, inhibitor screening, and selectivity profiling.

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 (H2A.Bbd) - biotinylated

Mononucleosomes (H2A.Bbd) were pulled down by streptavidin beads. Input mononucleosomes (Lane 1) and the mononucleosomes pulled down by streptavidin (Lane 2) were run on a 12.5% SDS-PAGE gel and stained with Coomassie Blue. The SDS-PAGE gel result shows that more than 80% biotinylated nucleosomes (H2A.Bbd) are pulled down by streptavidin beads. * indicates streptavidin.



Recombinant Mononucleosomes (H2A.Bbd) - biotinylated DNA gel

Biotinylated Mononucleosomes (H2A.Bbd) were run on a 2% agarose gel and stained with ethidium bromide. Lane 1: Intact mononucleosomes. Lane 2: 601 DNA which was used for assembly of mononucleosomes. Intact mononucleosomes migrate much higher than free DNA.

The agarose gel result shows that almost all of 601 DNA wrap histone octamers to form mononucleosomes.

DOT1L:	0.1	0	0.025	0.05	0.1	0.1	:ug
Nucleosomes:	0	2	2	2	2	0	
Histone Octamers:	0	0	0	0	0	2	



Western Blot analysis for Mononucleosomes (H2A.Bbd) - biotinylated

2 µg Recombinant Mononucleosomes (H2A.Bbd) were incubated with DOT1L (Cat# 31474) in reaction buffer for 3 h at room temperature. Western Blot was used for detecting the generation of reaction products (H3K79me1, Cat# 39921). DOT1L only and mononucleosomes only were used as negative control. The Western Blot result shows that mononucleosomes are more suitable substrate for DOT1L than histone octamers.