Recombinant Mononucleosomes (TH2B) - biotinylated

Catalog No: 31557, 31957
Expressed In: *E. coli*

Quantity: 20, 1000 µg
Concentration: 0.7 µg/µl
Source: Human

Buffer Contents: Recombinant Mononucleosomes (TH2B) - biotinylated (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. Please refer to product insert upon arrival for lot-specific concentration.

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 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 biology processes including chromosome segregation, DNA repair, transcriptional regulation and mRNA processing.

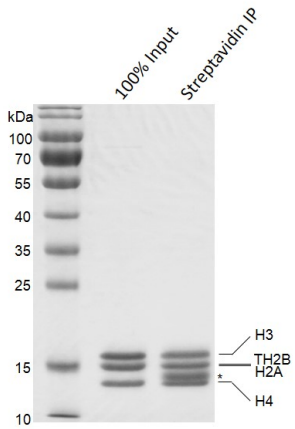
TH2B (testis-specific H2B) is originally identified as a testis-specific variant of histone H2B. TH2A is a testis-specific variant of histone H2A. They are abundant in the testis, oocytes and fertilized eggs. TH2A and TH2B are controlled by a shared promoter that is located between them. Disruption of *Th2a* and *Th2b* genes causes defects in spermatogenesis. TH2A/TH2B may enhance reprogramming by introducing processes that normally operate in zygotes and during SCNT. TH2A and TH2B induce nucleosome instability. TH2B controls the chromatin-to-nucleoprotamine transition.

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 (TH2B) - biotinylated, consist 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 NP_003503.1), TH2B that includes amino acids 1-127 (end) (accession number NP_733759.1), 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 ~108 kDa. The recombinant protein is >95% pure by SDS-PAGE.

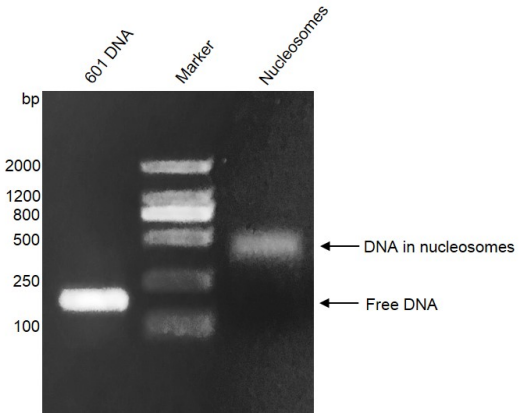
Application Notes: Recombinant Mononucleosomes (TH2B) - biotinylated are suitable for use as substrates in the study of enzyme kinetics, inhibitor screening, and selectivity profiling.

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 (TH2B) - biotinylated

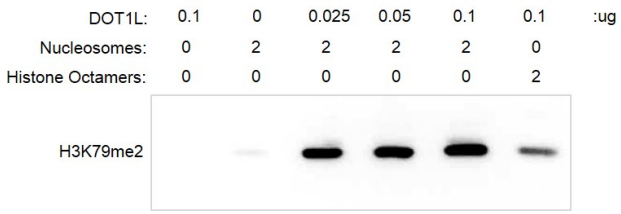
Mononucleosomes (TH2B) were pulled down by streptavidin beads. Input mononucleosomes (Lane 1) and the mononucleosomes pulled down by streptavidin (Lane 2) were run on a 12.5% SDS-PAGE gel and stained with Coomassie Blue.The SDS-PAGE gel result shows that more than 80% biotinylated nucleosomes (TH2B) are pulled down by streptavidin beads. * indicates streptavidin.



Recombinant Mononucleosomes (TH2B) - biotinylated DNA gel

Biotinylated Mononucleosomes (TH2B) were run on a 2% agarose gel and stained with ethidium bromide. Lane 1: 601 DNA which was used for assembly of nucleosomes. Lane 2: MW. Lane 3: Intact 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.



Western Blot analysis for Recombinant Mononucleosomes (TH2B) - biotinylated

2 µg Recombinant Mononucleosomes (TH2B) 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 (H3K79me2, Cat# 39143). DOT1L only and mononucleosomes only were used as negative control.

The Western Blot results show that mononucleosomes are a more suitable substrate for DOT1L than histone octamers.

Nucleosomes = Mononucleosomes.