

## Recombinant Mononucleosomes (H2A.X) - biotinylated

**Catalog No:** 31582, 31982

**Expressed In:** *E. coli*

**Quantity:** 20, 1000 µg

**Concentration:** 0.9 µg/µl

**Source:** Human

**Buffer Contents:** Recombinant Mononucleosomes (H2A.X) - biotinylated (20 µg protein + 19.2 µg DNA) are supplied at a protein concentration of 0.9 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 of four different types: H2A, H2B, H3 and H4. Histones are subject to posttranslational modifications, such as methylation, acetylation, phosphorylation, mono-ubiquitination, etc. 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.

H2A.X (also known as H2AFX, Histone Family Member X) is a histone H2A family member. In mammals, variant histone H2A.X represents 10% of the total H2A, whereas in yeast 90% of H2A is of the H2A.X class. The main function of H2AX is associated with the DNA damage repair (DDR) system which is induced by DNA double strand breaks (DSBs). In many species, including *S. cerevisiae*, *Xenopus laevis*, and *Drosophila melanogaster*, the fourth serine residue from the carboxyl-terminal end of histone H2A becomes phosphorylated. In mammals, this event is related to phosphorylation of the H2A.X variant histone on serine 139 by DNA-dependent protein kinases (ATR/ATM/DNA- PK). The phosphorylated form of H2A.X is commonly denoted as γ-H2A.X. This H2A.XS139 phosphorylation facilitates the recruitment of the DNA damage repair machinery, as well as chromatin remodelers such as INO80 and SWR1. H2A.X is a regulator of the epithelial–mesenchymal transition.

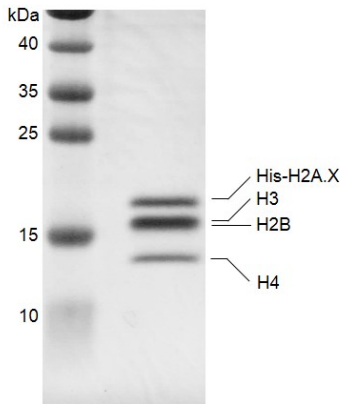
Nucleosomes are more physiologically relevant substrates than histones and histone-derived peptides for *in vitro* studies. More importantly, some histone methyltransferases are significantly more active, as well as specific, when using nucleosomal substrates in HMT assays, such as DOT1L and NSD family enzymes. Nucleosomes are also widely used in histone methyltransferase screening assays to identify small molecular inhibitors for drug discovery.

**Protein Details:** Recombinant Mononucleosomes (H2A.X) - biotinylated, consist of 167 bp of 601 DNA with a 5' biotin tag and two molecules each of histones H2A.X that includes amino acids 1-143 (end) (accession number NP\_002096.1) with a C-terminal 6×His tag, 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* cells. The molecular weight of the histone octamer is ~113.2 kDa.

**Application Notes:** Recombinant Mononucleosomes (H2A.X) - 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

### Mononucleosomes (H2A.X)

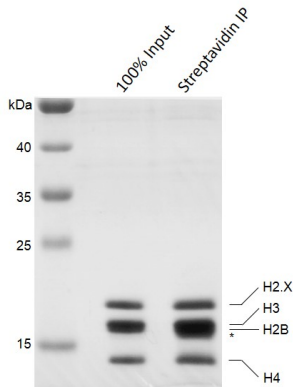


### Recombinant Mononucleosomes (H2A.X) - biotinylated, SDS-PAGE.

12.5% SDS-PAGE gel stained with Coomassie Blue.

Purity:  $\geq 95\%$

### Nucleosomes (H2A.X)



### Streptavidin pull-down of Recombinant Mononucleosomes (H2A.X) - biotinylated.

Mononucleosomes (H2A.X) were pulled down with 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 gel result shows that almost all biotinylated mononucleosomes (H2A.X) are pulled down by Streptavidin beads. \*Indicates Streptavidin.

Nucleosomes = Mononucleosomes

### Recombinant Mononucleosomes (H2A.X) - biotinylated, DNA gel.

Biotinylated mononucleosomes (H2A.X) were run on a 2% agarose gel and stained with ethidium bromide. Lane 1: DNA marker. Lane 2: free 601 DNA. Lane 3: Intact mononucleosomes. Intact mononucleosomes migrate much higher than free 601 DNA. The agarose gel result shows that almost all of 601 DNA wraps histone octamers to form mononucleosomes.

