Mononucleosomes (H3.3K27M), Recombinant Human, Biotinylated

Catalog No 16-0323
Lot No 21214004-07
Pack Size 100 µg

Product Description:

Mononucleosomes assembled from recombinant human histones expressed in *E. coli* (two each of histones H2A, H2B, H3.3 and H4; accession numbers: H2A-P04908; H2B-O60814; H3.3-P84243; H4-P62805) wrapped by 147 base pairs of 601 positioning sequence DNA. Histone H3.3 is a non-allelic replacement found in regions of high chromatin turnover outside of S-phase (e.g. at actively transcribed genes). The substitution of M for K at position 27 is a dominant negative inhibitor of the EZH2 methyltransferase complex PRC2 and is associated with pediatric brain cancer (DIPG) [1]. The nucleosome is the basic subunit of chromatin. The 147 bp 601 sequence, identified by Lowary and Widom [2], has high affinity for histone octamers and is useful for nucleosome assembly. The DNA contains a 5' biotin-TEG group.

Formulation:

H3.3K27M Mononucleosomes (54.6 µg protein weight, 100.0 µg DNA + protein) in 90.9 µL 10 mM Tris HCl pH 7.5, 25 mM NaCl, 1 mM EDTA, 2 mM DTT, 20% glycerol. Molarity = 5.5 µM. MW = 199,844 Da.

Storage and Stability:

Stable for six months at -80°C from date of receipt. For best results, aliquot and avoid multiple freeze/thaws.

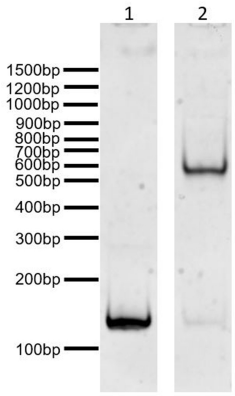
Application Notes:

H3.3K27M Mononucleosomes is highly purified and suitable for a variety of applications, including use as a substrate in enzymatic assays or for effector protein binding experiments.

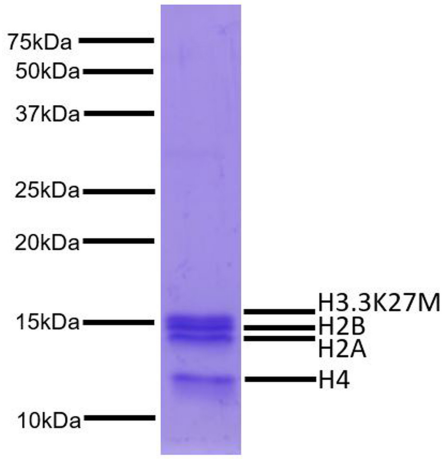
References:

[1] Chan et al. (2013) *Genes Dev* 27:985-990.
[2] Lowary PT and Widom J (1998) *J Mol Biol* 276:19-42.

Western Blot Data: Western analysis of Mononucleosomes (H3.3K27M), Recombinant Human. **Top Panel:** Wild-type H3.3 (Lane 1) and H3.3K27M nucleosomes (Lane 2) were probed with an H3K27M antibody. Only the H3.3K27M sample produced a detectable signal. **Bottom Panel:** Detail from Coomassie stained gel of Western blot.



DNA Gel Data: H3.3K27M Mononucleosomes resolved via Native PAGE and stained with ethidium bromide to visualize DNA. **Lane 1:** Free DNA extracted from nucleosomes (100 ng). **Lane 2:** Intact nucleosomes (400 ng).



Protein Gel Data: Coomassie stained PAGE gel of proteins in H3.3K27M Mononucleosomes (1 µg) demonstrates the purity of histones in the preparation. Sizes of molecular weight markers and positions of the core histones (H2A, H2B, H3.3K27M and H4) are indicated.