Histone H3.1
Human, Recombinant

Source: An E. coli strain that carries a plasmid encoding the cloned human histone H3.1 gene, HIST1H3A or H3FA. (Genbank accession number: AF531274)

Supplied in: 20 mM Sodium Phosphate (pH 7.0), 300 mM NaCl, 1 mM EDTA and 1 mM DTT.

Note: The protein concentration (1 mg/ml, 65 µM) is calculated using the molar extinction coefficient for Histone H3.1 (4080) and its absorbance at 280 nm (3.4). 1.0 A280 units = 3.8 mg/ml


Quality Control Assays:
SDS-PAGE: 0.5, 1.0, 2.0, 5.0, 10.0 µg of Histone H3.1 Human, Recombinant were loaded on a 10–20% Tris-Glycine SDS-PAGE gel and stained with Coomassie Blue. The calculated molecular weight is 15272.89 Da. Its apparent molecular weight on 10–20% Tris-Glycine SDS-PAGE gel is ~15 kDa.

Mass Spectrometry: The mass of purified Histone H3.1 Human, Recombinant is 15273.2 Da as determined by ESI-TOF MS (Electrospray Ionization-Time of Flight Mass Spectrometry). The average mass calculated from primary sequence is 15272.89 Da. This confirms the protein identity as well as the absence of any modifications of the histone.

N-terminal Protein Sequencing: Protein identity was confirmed using Edman Degradation to sequence the intact protein.

Enzyme Modification:
1. G9a Methyltransferase: After incubation of a 25 µl reaction for 10 minutes at 37°C, 1 unit of G9a methyltransferase (NEB #M0235) transfers 0.6 pmols of methyl group to Histone H3.1 Human, Recombinant.
2. SET7 Methyltransferase: After incubation of a 25 µl reaction for 10 minutes at 37°C, 1 unit of SET7 methyltransferase (NEB #M0233) transfers 1 pmol of methyl group to Histone H3.1 Human, Recombinant.

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Protease Assay: After incubation of 10 µg of Histone H3.1 Human, Recombinant with a standard mixture of proteins for 2 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Exonuclease Assay: Incubation of a 50 µl reaction containing 10 µg of Histone H3.1 Human, Recombinant with 1 µg of a mixture of single and double-stranded [³H] E. coli DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Assay: Incubation of a 50 µl reaction containing 10 µg of Histone H3.1 Human, Recombinant with 1 µg of φX174 RF I (suprecoiled) plasmid DNA for 4 hours at 37°C resulted in < 5.0% conversion to RF II form (nicked circle) as determined by agarose gel electrophoresis.

Protein Sequence: ARTKQTARKSTGGKAPRK QLATKAARKSAPATGGVKHPHRPGTVALEI RRYQKSTELLIRKLPQORLREIAQDKTLRFQ SSAMOACAEAVLVQNLCAHAKRVT IMPKIQLARRIRGERA (Genbank accession number: AAN10051)

Usage Note: When running SDS-PAGE gels, Histone H3.1 can self-oligomerize if freshly prepared DTT (dithiothreitol) is not used when preparing the sample for gel electrophoresis

References: